(12) **UK Patent Application** (19) **GB** (11) **2 268 744** (13) **A**

(21) Application No 9318911.6

(22) Date of Filing 21.12.1990

Date Lodged 13.09.1993

(30) Priority Data
(31) 8928874 .   (32) 21.12.1989   (33) **GB** ·

(62) Derived from Application No. 9117611.5 under Section 15(4) of the Patents Act 1977

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(51) INT CL⁵
C12P 21/08 , A61K 39/395 49/00 , C07K 15/06 , C12N 15/13 15/62 // C12N 5/10 (C12N 5/10 C12R 1:91)

(52) UK CL (Edition M)
C3H HB7M H656 H675 H690
C6Y Y406 Y407 Y501 Y503
U1S S1337 S2419

(56) Documents Cited
None

(58) Field of Search
UK CL (Edition L) **C3H HB7M**
INT CL⁵ C07K 15/06 15/12 , C12N 15/13 , C12P 21/08

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(54) Recombinant antibody having anti-CD3 binding specificity

(57) A recombinant antibody molecule comprises antigen binding regions derived from the heavy and/or light chain variable regions of a donor anti-CD3 antibody, e.g. OKT3, and has anti-CD3 binding specificity, preferably of affinity similar to that of OKT3. The recombinant antibody is preferably a humanised antibody and may be a chimeric or CDR-grafted antibody. A protocol is disclosed for preparing CDR-grafted humanised antibodies in which, in addition to the CDRs non-human antibody residues are preferably used at positions 23, 24, 49, 71, 73 and 78 of the heavy chain variable region and at positions 46, 48, 58 and 71 of the light chain variable region. The recombinant, especially the humanised, anti-CD3 antibodies may be used for in vivo therapy or diagnosis.

The date of filing shown above is that provisionally accorded to the application in accordance with the provisions of Section 15(4) of the Patents Act 1977 and is subject to ratification or amendment.

At least one of these pages has been prepared from an original which was unsuitable for direct photoreproduction.

GB 2 268 744 A

```
  1 GAATTCCCAA AGACAAAatg cattttcaac tccacatttt cagcttcctc
 51 ctaatcactc cctcagtcat aatatccaga ggacaaattg ttctcaccca
101 gtctccagca atcatgtctg catctccagg ggacaaggtc accatgacct
151 gcagtgccag ctcaagtgta agttacatga ctggtacca gcacaagtca
201 ggcacctccc ccaaaagatg gatttatgac acatccaaac tggcttctgg
251 agtccctgct cacttcaggg gcagtgggtc tgggacctct tactctctca
301 caatcagcgg catggaggct gaagatgctg ccacttatta ctgccagcag
351 tggagtagta acccattcac gttcggctcg gggacaaagt tggaaataaa
401 ccgggctgat actgcaccaa ctgtatccat cttcccacca tccagtgagc
451 agttaacatc tggaggtgcc tcagtcgtgt gcttcttgaa caacttctac
501 cccaaagaca tcaatgtcaa gtggaagatt gatggcagtg aacgacaaaa
551 tggcgtcctg aacagttgga ctgatcagga cagcaaagac agcacctaca
601 gcatgagcag caccctcacg ttgaccaagg acgagtatga acgacataac
651 agctatacct gtgaggccac tcacaagaca tcaacttcac ccattgtcaa
701 gagcttcaac aggaatgagt gtTAGAGACA AAGGTCCTGA GACGCCACCA
751 CCAGCTCCCA GCTCCATCCT ATCTTCCCTT CTAAGGTCTT GGAGGCTTCC
801 CCACAAGCGC tTACCACTGT TGCGGTGCTC tAAACCTCCT CCCACCTCCT
851 TCTCCTCCTC CTCCCTTTCC TTGGCTTTTA TCATGCTAAT ATTTGCAGAA
901 AATATTCAAT AAAGTGAGTC TTTGCCTTGA AAAAAAAAA AAA
```

Fig 1(a)

```
  1 MDFQVQIFSF LLISASVIIS RGQIVLTQSP AIMSASPGEK VTMTCSASSS
 51 VSYMNWYQQK SGTSPKRWIY DTSKLASGVP AHFRGSGSGT SYSLTISGME
101 AEDAATYYCQ QWSSNPFTFG SGTKLEINRA DTAPTVSIFP PSSEQLTSGG
151 ASVVCFLNNF YPKDINVKWK IDGSERQNGV LNSWTDQDSK DSTYSMSSTL
201 TLTKDEYERH NSYTCEATHK TSTSPIVKSF NRNEC*
```

Fig 1(b)

Fig 2(a)

```
   1  GAATTCCCCT CTCCACAGAC ACTGAAAACT CTGACTCAAC ATGGAAAGGC
  51  ACTGGATCTT TCTACTCCTG TTGTCAGTAA CTGCAGGTGT CCACTCCCAG
 101  GTCCAGCTGC AGCAGTCTGG GGCTGAACTG GCAAGACCTG GGGCCTCAGT
 151  GAAGATGTCC TGCAAGGCTT CTGGCTACAC CTTTACTAGG TACACGATGC
 201  ACTGGGTAAA ACAGAGGCCT GGACAGGGTC TGGAATGGAT TGGATACATT
 251  AATCCTAGCC GTGGTTATAC TAATTACAAT CAGAAGTTCA AGGACAAGGC
 301  CACATTGACT ACAGACAAAT CCTCCAGCAC AGCCTACATG CAACTGAGCA
 351  GCCTGACATC TGAGGACTCT GCAGTCTATT ACTGTGCAAG ATATTATGAT
 401  GATCATTACT GCCTTGACTA CTGGGGCCAA GGCACCACTC TCACAGTCTC
 451  CTCAGCCAAA ACAACAGCCC CATCGGTCTA TCCACTGGCC CCTGTGTGTG
 501  GAGATACAAC TGGCTCCTCG GTGACTCTAG GATGCCTGGT CAAGGGTTAT
 551  TTCCCTGAGC CAGTGACCTT GACCTGGAAC TCTGGATCCC TGTCCAGTGG
 601  TGTGCACACC TTCCCAGCTG TCCTGCAGTC TGACCTCTAC ACCCTCAGCA
 651  GCTCAGTGAC TGTAACCTCG AGCACCTGGC CCAGCCAGTC CATCACCTGC
 701  AATGTGGCCC ACCCGGCAAG CAGCACCAAG GTGGACAAGA AAATTGAGCC
 751  CAGAGGGCCC ACAATCAAGC CCTGTCCTCC ATGCAAATGC CCAGCACCTA
 801  ACCTCTTGGG TGGACCATCC GTCTTCATCT TCCCTCCAAA GATCAAGGAT
 851  GTACTCATGA TCTCCCTGAG CCCCATAGTC ACATGTGTGG TGGTGGATGT
 901  GAGCGAGGAT GACCCAGATG TCCAGATCAG CTGGTTTGTG AACAACGTGG
 951  AAGTACACAC AGCTCAGACA CAAACCCATA GAGAGGATTA CAACAGTACT
1001  CTCCGGGTGG TCAGTGCCCT CCCCATCCAG CACCAGGACT GGATGAGTGG
1051  CAAGGAGTTC AAATGCAAGG TCAACAACAA AGACCTCCCA GCGCCCATCG
1101  AGAGAACCAT CTCAAAACCC AAAGGGTCAG TAAGAGCTCC ACAGGTATAT
1151  GTCTTGCCTC CACCAGAAGA AGAGATGACT AAGAAACAGG TCACTCTGAC
1201  CTGCATGGTC ACAGACTTCA TGCCTGAAGA CATTTACGTG GAGTGGACCA
1251  ACAACGGGAA AACAGAGCTA AACTACAAGA ACACTGAACC AGTCCTGGAC
1301  TCTGATGGTT CTTACTTCAT GTACAGCAAG CTGAGAGTGG AAAAGAAGAA
1351  CTGGGTGGAA AGAAATAGCT ACTCCTGTTC AGTGGTCCAC GAGGGTCTGC
1401  ACAATCACCA CACGACTAAG AGCTTCTCCC GGACTCCGGG TAAATGAGCT
1451  CAGCACCCAC AAAACTCTCA GGTCCAAAGA GACACCCACA CTCATCTCCA
1501  TGCTTCCCTT GTATAAATAA AGCACCCAGC AATGCCTGGG ACCATGTAAA
1551  AAAAAAAAAA AAAGGAATTC
```

OKT3 HEAVY CHAIN PROTEIN SEQUENCE DEDUCED FROM DNA
SEQUENCE

Fig 2(b)

```
  1   MERHWIFILL LSVTAGVESQ VQLQQSGAEL ARPGASVKMS CKASGYTFTR
 51   YTMHWVKQRP GQGLEWIGYI NPSRGYTNYN QKFKDKATLT TDKSSSTAYM
101   QLSSLTSEDS AVYYCARYYD DHYCLDYWGQ GTTLTVSSAK TTAPSVYPLA
151   PVCGDTTGSS VTLGCLVKGY FPEPVTLTWN SGSLSSGVHT FPAVLQSDLY
201   TLSSSVTVTS STWPSQSITC NVAHPASSTK VDKKIEPRGP TIKPCPPCKC
251   PAPNLLGGPS VFIFPPKIKD VLMISLSPIV TCVVVDVSED DPDVQISWFV
301   NNVEVHTAQT QTHREDYNST LRVVSALPIQ HQDWMSGKEF KCKVNNKDLP
351   APIERTISKP KGSVRAPQVY VLPPPEEEMT KKQVTLTCMV TDFMPEDIYV
401   EWTNNGKTEL NYKNTEPVLD SDGSYFMYSK LRVEKKNWVE RNSYSCSVVH
451   EGLHNHHTTK SFSRTPGK*
```

Fig 3

```
                 1                        23                      42
                 NN       N                  N          N      N
RES TYPE    SBspSPESssESbSsSssPSPSPsPSsse*s*p*Pi^ISsSe
Okt3v1      QIVLTQSPAIMSASPGEKVTMTCSASS.SVSYMNWYQQKSGT
REI         DIQMTQSPSSLSASVGDRVTITCQASQDIIKYLNWYQQTPGK
                 ? ?
                    CDR1   (LOOP)              *******
                    CDR1   (KABAT)          **********


                             56                              85
                 N  NN
RES TYPE    *IsiPpIeesessssSBEsePsPSBSSEsPspsPsseesSPePb
Okt3v1      SPKRWIYDTSKLASGVPAHFRGSGSGTSYSLTISGMEAEDAAT
REI         APKLLIYEASNLQAGVPSRFSGSGSGTDYTFTISSLQPEDIAT
                 ? ??                                   ?  ?
                      ****** CDR2   (LOOP/KABAT)


                             102    108
RES TYPE    PiPIPies**iPIIsPPSPSPSS
Okt3v1      YYCQQWSSNPFTFGSGTKLEINR
REIv1       YYCQQYQSLPYTFGQGTKLQITR
                              ?       ?
                    ******          CDR3   (LOOP)
                    ********        CDR3   (KABAT)
```

Fig 4

```
                     NN  N                          23 26      32  35  N39    43
RES TYPE    SESPs^SEssS^sSSsSpSpSPsPSEbSEssBePiPIpiesss
Okt3vh      QVQLQQSGAELARPGASVKMSCKASGYTFTRYTMHWVKQRPGQ
KOL         QVQLVESGGGVVQPGRSLRLSCSSSGFIFSSYAMYWVRQAPGK
                     ?                                              ??

                                                      * * * * * *       CDR1  (LOOP)
                                                        * * * * *       CDR1  (KABAT)


                     52a          60  65        N  N   N      82abc        89
RES TYPE    IIeIppp^ssssssss^ps^pSSsbSpseSsSseSp^pSpsSEssS^ePb
Okt3vh      GLEWIGYINPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLTSEDSAV
KOL         GLEWVAIIWDDGSDQHYADSVKGRFTISRDNSKNTLFLQMDSLRPEDTGV
                     ??                                   ? ?   ?  ?
                                                                              ?
                     * * * * * * * * * *    CDR2  (LOOP)
                     * * * * * * * * * * * * * * * * *    CDR2  (KABAT)


                     92 N                        107    113
RES TYPE    PiPIEissssiiiisssbibi*EIPIP*spSBSS
Okt3vh      YYCARYYDDHY.......CLDYWGQGTTLTVSS
KOL         YFCARDGGHGFCSSASCFGPDYWGQGTPVTVSS
                     * * * * * * * * * * * * * * *    CDR3  (KABAT/LOOP)
```

OKT 3 HEAVY CHAIN CDR GRAFTS

1. gh341 and derivatives

```
               1                              26        35  39  43
Okt3vh    QVQLQQSGAELARPGASVKMSCKASGYTFTRYTMHWVKQRPGQ
gH341     QVQLVESGGGVVQPGRSLRLSCSSSGYTFTRYTMHWVRQAPGK      JA178
gH341A    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK      JA185


gH341E    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK      JA198
gH341*    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK      JA207
gH341*    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK      JA209
gH341D    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK      JA197
gH341*    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK      JA199
gH341C    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK      JA184


gH341*    QVQLVQSGGGVVQPGRSLRLSCSASGYTFTRYTMHWVRQAPGK      JA203
gH341*    QVQLVESGGGVVQPGRSLRLSCSASGYTFTRYTMHWVRQAPGK      JA205
gH341B    QVQLVESGGGVVQPGRSLRLSCSSSGYTFTRYTMHWVRQAPGK      JA183
gH341*    QVQLVQSGGGVVQPGRSLRLSCSASGYTFTRYTMHWVRQAPGK      JA204
gH341*    QVQLVESGGGVVQPGRSLRLSCSASGYTFTRYTMHWVRQAPGK      JA206
gH341*    QVQLVQSGGGVVQPGRSLRLSCSASGYTFTRYTMHWVRQAPGK      JA208
KOL       QVQLVESGGGVVQPGRSLRLSCSSSGFIFSSYAMYWVRQAPGK
```

Fig 5 (i)

```
                44      50                    65                              83
      Okt3vh    GLEWIGYINPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLT
      gH341     GLEWVAYINPSRGYTNYNQKFKDRFTISRDNSKNTLFLQMDSLR  JA178
      gH341A    GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKSTAFLQMDSLR  JA185

      gH341E    GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKSTAFLQMDSLR  JA198
      gH341*    GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKNTAFLQMDSLR  JA207
      gH341*    GLEWIGYINPSRGYTNYNQKVKDRFTISRDNSKNTAFLQMDSLR  JA209
      gH341D    GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKNTLFLQMDSLR  JA197
      gH341*    GLEWIGYINPSRGYTNYNQKVKDRFTISRDNSKNTLFLQMDSLR  JA199
      gH341C    GLEWVAYINPSRGYTNYNQKFKDRFTISRDNSKNTLFLQMDSLR  JA184

      gH341*    GLEWIGYINPSRGYTNYNOKVKDRFTISTDKSKSTAFLQMDSLR  JA207
      gH341*    GLEWIGYINPSRGYTNYNOKVKDRFTISTDKSKSTAFLQMDSLR  JA205
      gH341B    GLEWIGYINPSRGYTNYNOKVKDRFTISTDKSKSTAFLQMDSLR  JA183
      gH341*    GLEWIGYINPSRGYTNYNOKVKDRFTISTDKSKSTAFLQMDSLR  JA204
      gH341*    GLEWIGYINPSRGYTNYNOKVKDRFTISTDKSKSTAFLQMDSLR  JA206
      gH341*    GLEWIGYINPSRGYTNYNOKVKDRFTISTDKSKNTAFLQMDSLR  JA208
      KOL       GLEWVAIIWDDGSDQHYADSVKGRFTISRDNSKNTLFLQMDSLR
```

Fig 5 (ii)

```
                84              95                102            113
Okt3vh        SEDSAVYYCARYYDDHY.......CLDYWGQGTTLTVSS
gH341         PEDTGVYFCARYYDDHY......CLDYWGQGTTLTVSS        JA178
gH341A        PEDTAVYYCARYYDDHY......CLDYWGQGTTLTVSS        JA185

gH341E        PEDTGVYFCARYYDDHY......CLDYWGQGTTLTVSS        JA198
gH341*        PEDTGVYFCARYYDDHY......CLDYWGQGTTLTVSS        JA207
gH341D        PEDTGVYFCARYYDDHY......CLDYWGQGTTLTVSS        JA197
gH341*        PEDTGVYFCARYYDDHY......CLDYWGQGTTLTVSS        JA209
gH341*        PEDTGVYFCARYYDDHY......CLDYWGQGTTLTVSS        JA199
gH341C        PEDTGVYFCARYYDDHY......CLDYWGQGTTLTVSS        JA184

gH341*        PEDTAVYYCARYYDDHY......CLDYWGQGTTLTVSS        JA203
gH341*        PEDTAVYYCARYYDDHY......CLDYWGQGTTLTVSS        JA205
gH341B        PEDTAVYYCARYYDDHY......CLDYWGQGTTLTVSS        JA183
gH341*        PEDTGVYFCARYYDDHY......CLDYWGQGTTLTVSS        JA204
gH341*        PEDTGVYFCARYYDDHY......CLDYWGQGTTLTVSS        JA206
gH341*        PEDTGVYFCARYYDDHY......CLDYWGQGTTLTVSS        JA208
KOL           PEDTGVYFCARDGGHGFCSSASCFGPDYWGQGTPVTVSS
```

Fig. 5(iii)

OKT3 LIGHT CHAIN CDR GRAFTING

1. gL221 and derivatives

```
              1                          24        34        42
Okt3vl   QIVLTQSPAIMSASPGEKVTMTCSASS.SVSYMNWYQQKSGT
gL221    DIQMTQSPSSLSASVGDRVTITCSASS.SVSYMNWYQQTPGK
gL221A   QIVMTQSPSSLSASVGDRVTITCSASS.SVSYMNWYQQTPGK
gL221B   QIVMTQSPSSLSASVGDRVTITCSASS.SVSYMNWYQQTPGK
gL221C   DIQMTQSPSSLSASVGDRVTITCSASS.SVSYMNWYQQTPGK
REI      DIQMTQSPSSLSASVGDRVTITCQASQDIIKYLNWYQQTPGK
```

```
         43        50        56                          85
Okt3vl   SPKRWIYDTSKLASGVPAHFRGSGSGTSYSLTISGMEAEDAAT
gL221    APKLLIYDTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIAT
gL221A   APKRWIYDTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIAT
gL221B   APKRWIYDTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIAT
gL221C   APKRWIYDTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIAT
REI      APKLLIYEASNLQAGVPSRFSGSGSGTDYTFTISSLQPEDIAT
```

```
         86    91    96          108
Okt3vl   YYCQQWSSNPFTFGSGTKLEINR
gL221    YYCQQWSSNPFTFGQGTKLQITR
gL221A   YYCQQWSSNPFTFGQGTKLQITR
gL221B   YYCQQWSSNPFTFGQGTKLQITR
gL221C   YYCQQWSSNPFTFGQGTKLQITR
REI      YYCQQYQSLPYTFGQGTKLQITR
```

CDR'S ARE UNDERLINED

FRAMEWORK RESIDUES INCLUDED IN THE GENE ARE DOUBLE UNDERLINED

Fig. 6

Figure 7

OKT3 - pJA198 EVALUATION
BINDING ASSAY
(Mean Channel - HPBALL's)
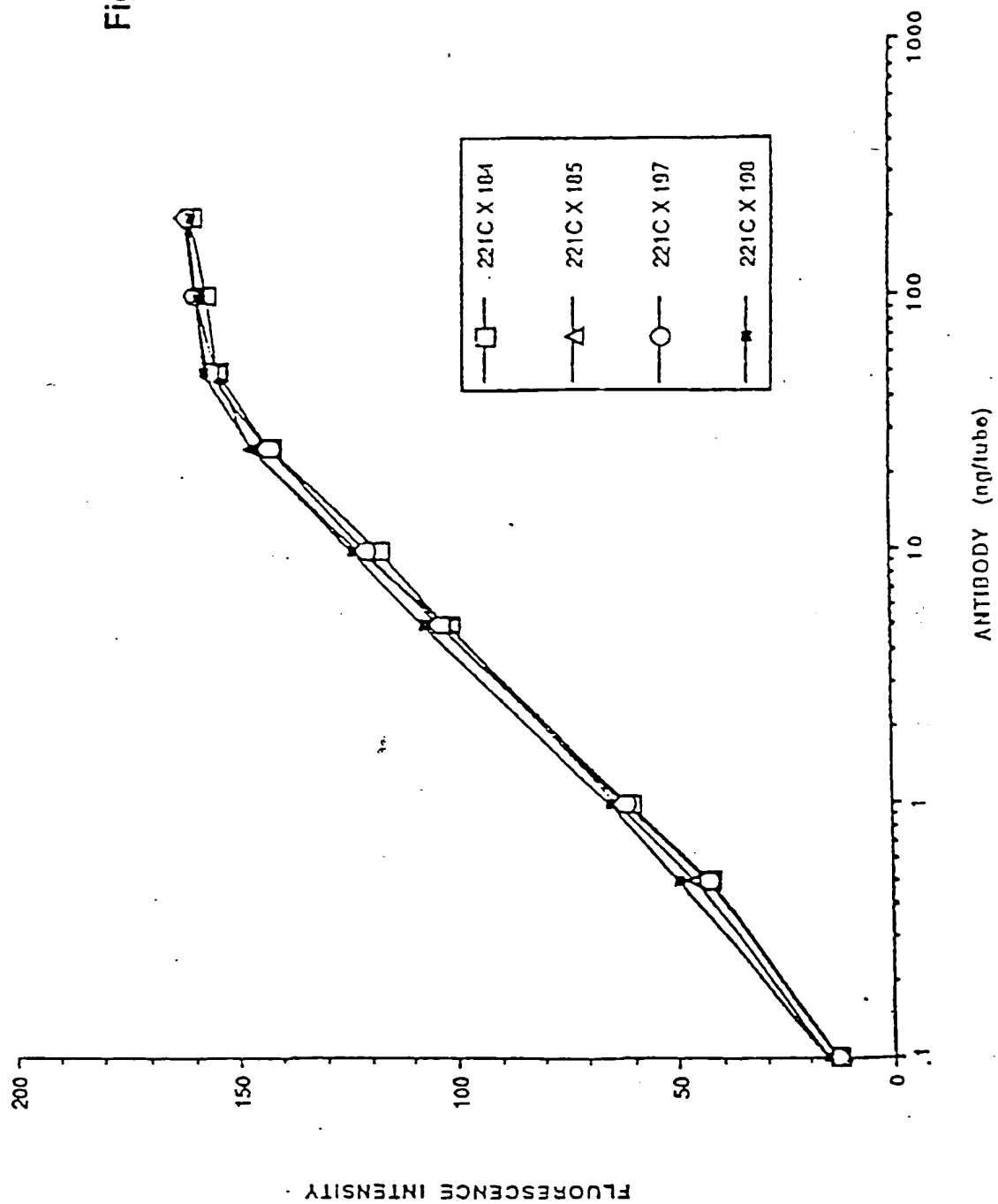
Figure 8

OKT3 - pJA198 EVALUATION
BLOCKING ASSAY
(Mean Channel -HPBALL's)
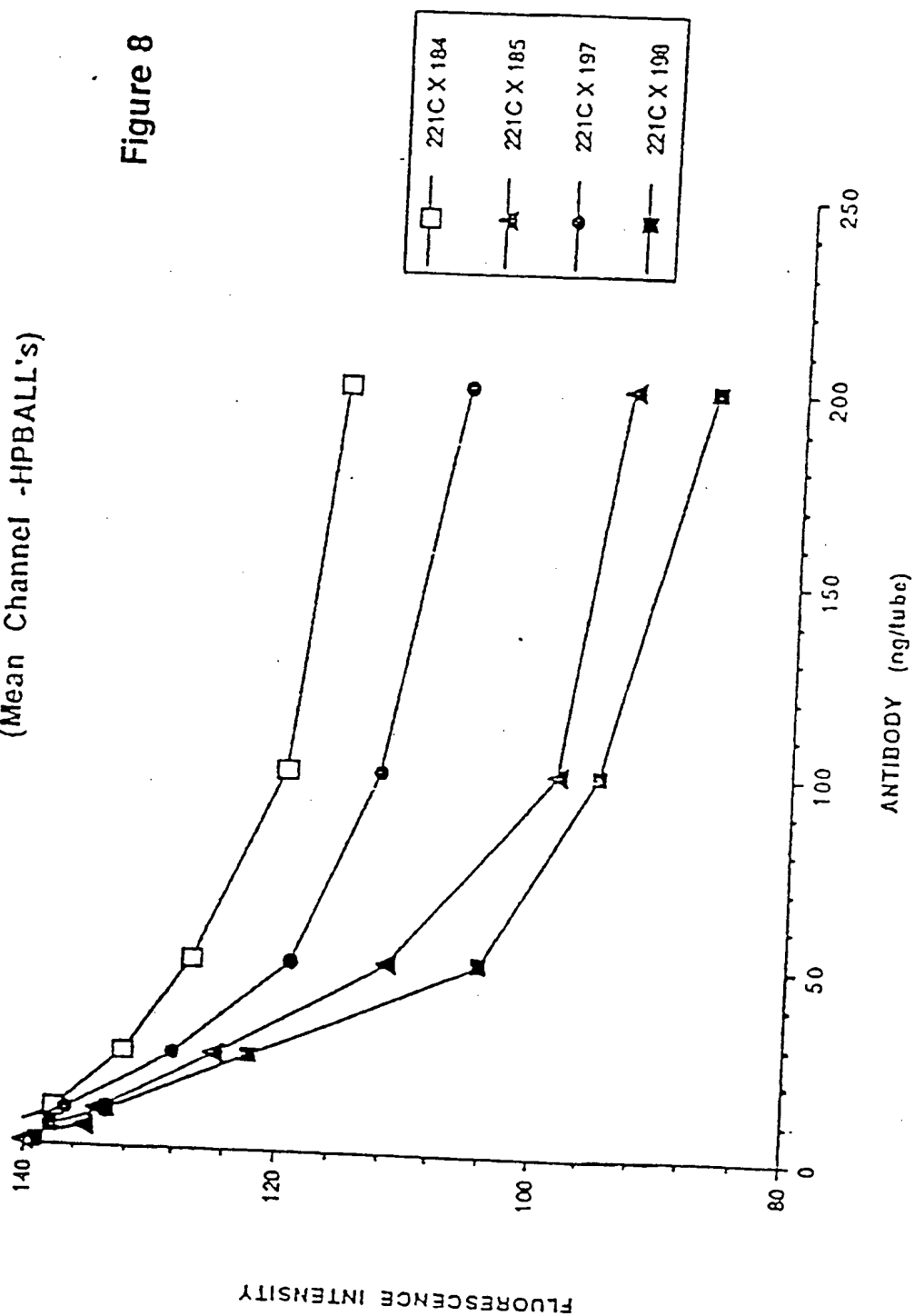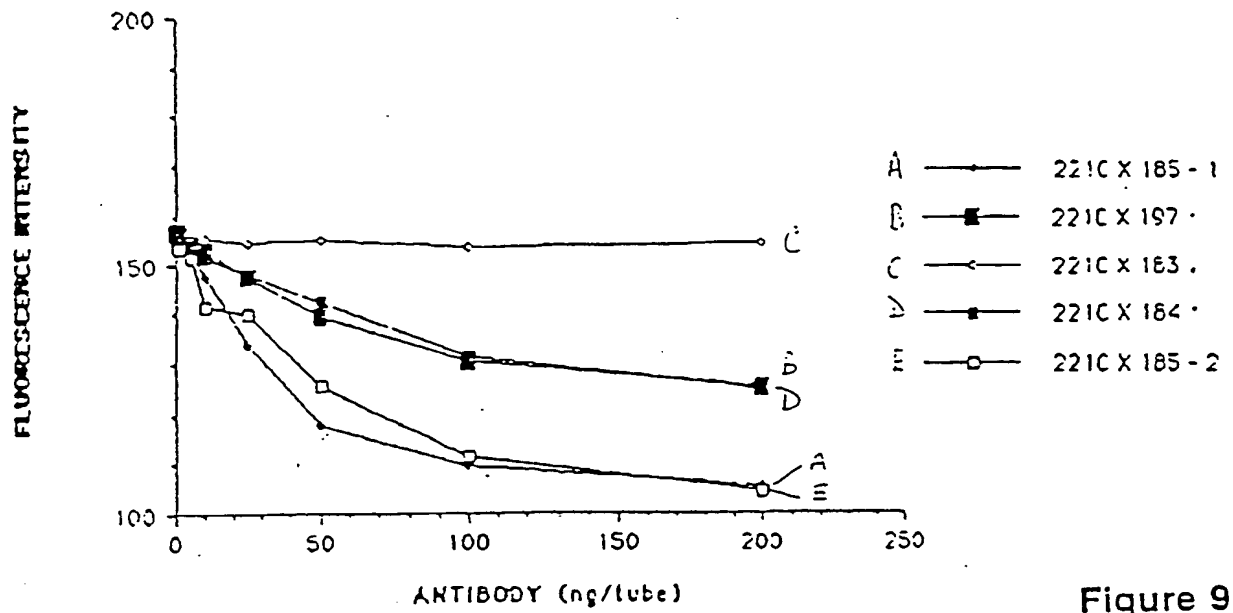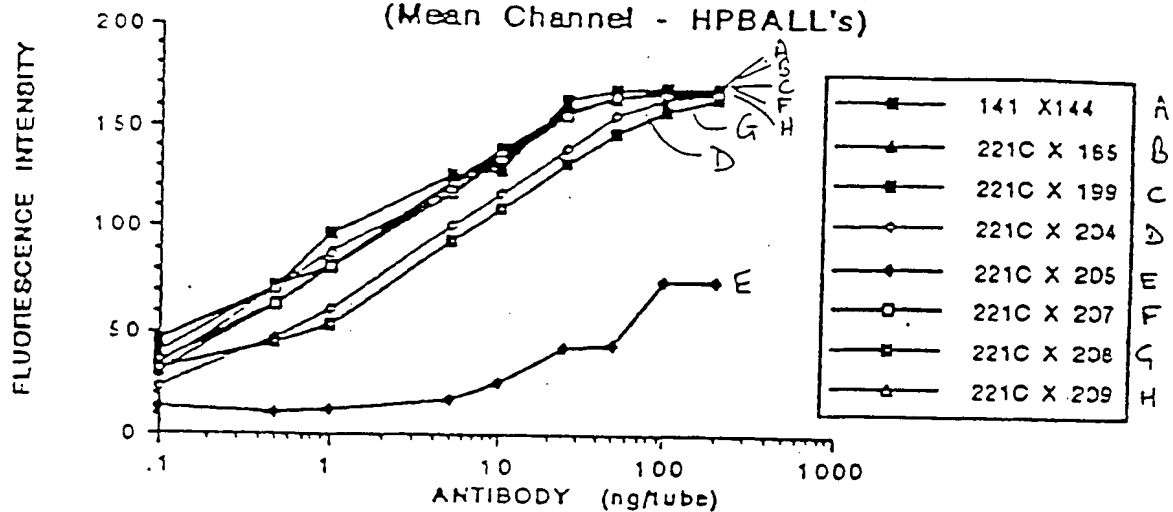
BLOCKING ASSAY
(Mean Channel – HPBALL's)

Figure 9

OKT3 - GRAFTED HEAVY CHAINS
BINDING ASSAY
(Mean Channel - HPBALL's)

FLUORESCENCE INTENSITY

200

150

100

50

0

.1 1 10 100 1000

ANTIBODY (ng/tube)

	141 X 144	A
	221C X 165	B
	221C X 199	C
	221C X 204	D
	221C X 205	E
	221C X 207	F
	221C X 208	G
	221C X 209	H

OKT3 - GRAFTED HEAVY CHAINS
BLOCKING ASSAY
(Mean Channel - HPBALL's)

FLUORESCENCE INTENSITY

200

150

100

50

0

0 50 100 150 200 250

ANTIBODY (ng/tube)

	141 X 144	A
	221C X 165	B
	221C X 199	C
	221C X 204	D
	221C X 205	E
	221C X 207	F
	221C X 208	G
	221C X 209	H

Figure 10

	(205)	_,__,24,48,49,71,73,76,78,88,91,
	(206)	6,__,24,48,49,71,73,__,78,__,__,
	(204)	6,__,24,48,49,71,73,76,78,__,__,
	(199)	6,23,24,48,49,__,__,__,__,__,
	(207)	6,23,24,48,49,71,73,__,78,__,__,
	(165)	6,23,24,48,49,71,73,76,78,88,91,
	(209)	6,23,24,48,49,__,__,__,78,__,__,
	141 X 144	

OKT3 - GRAFTED HEAVY CHAINS
BINDING ASSAY
(Mean Channel - HPBALL's)

OKT3 - GRAFTED HEAVY CHAINS
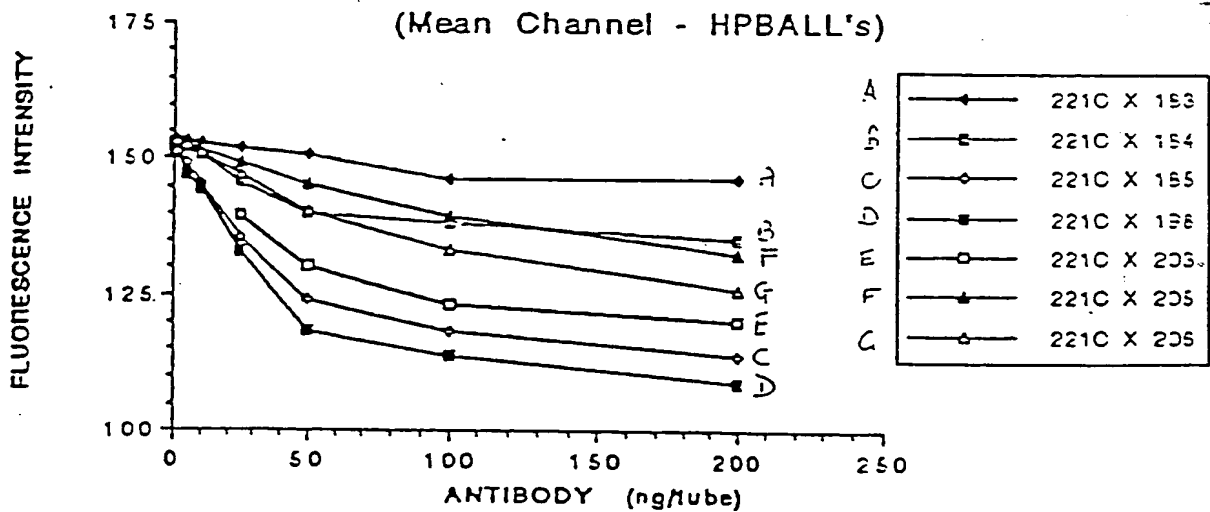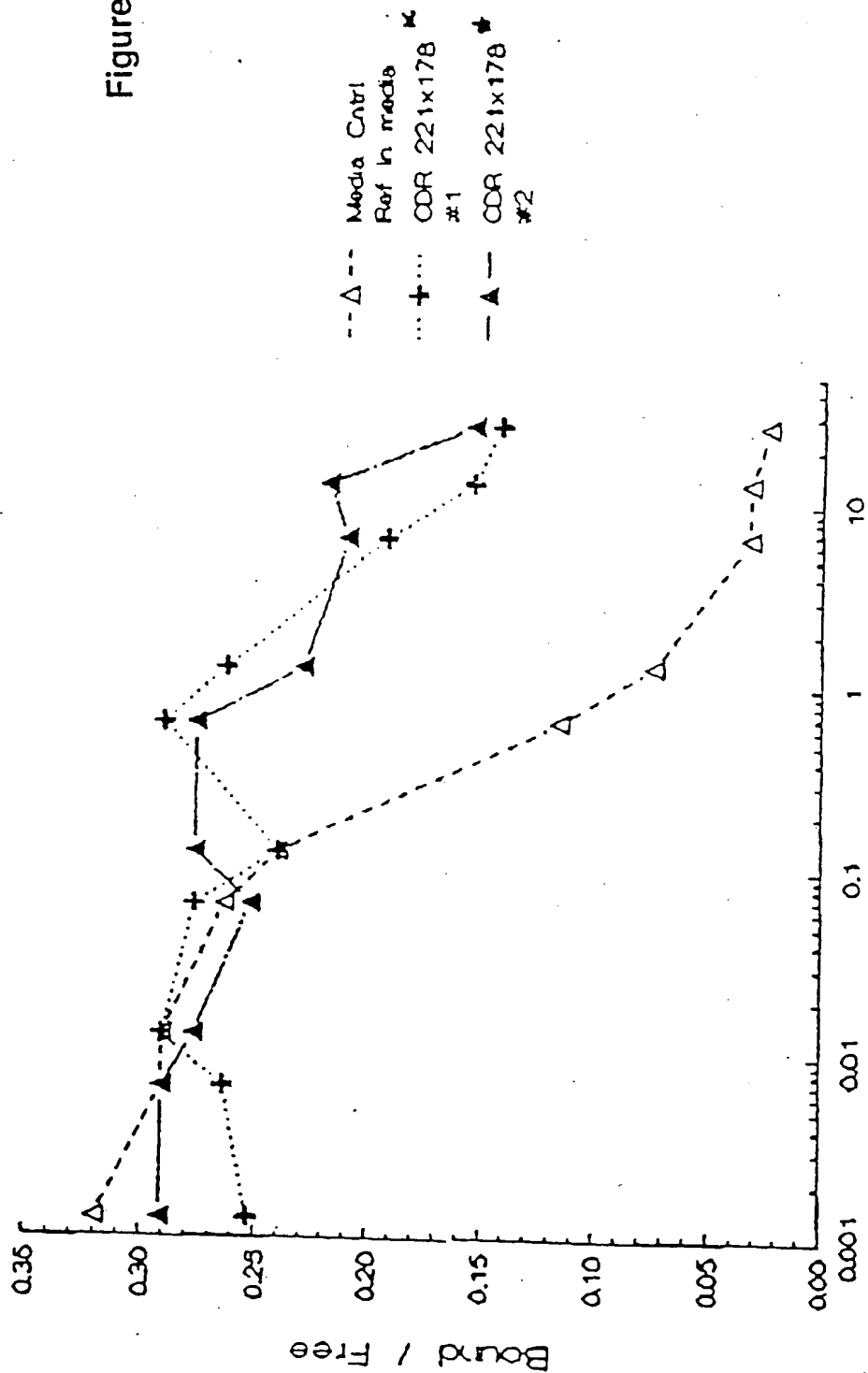BLOCKING ASSAY
(Mean Channel - HPBALL's)

Figure 11

Figure 12

OKT3 Competition
Murine Ref Std vs. CDR Grafted OKT3

OKT3 Competition
Murine Ref Std vs. CDR Grafted OKT3

Figure 13
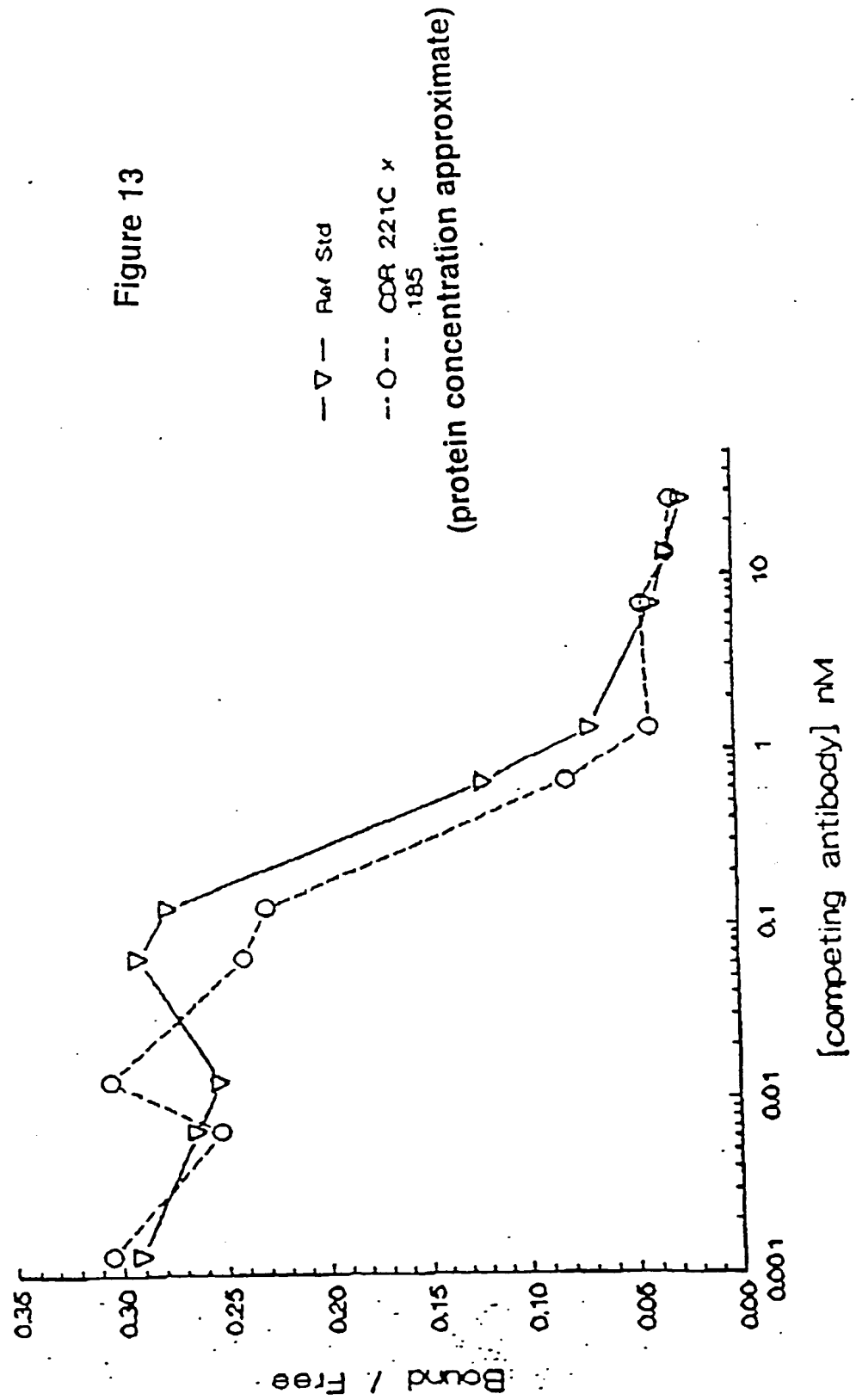
RECOMBINANT ANTIBODY AND METHOD

Field of the Invention

The present invention relates to a recombinant antibody molecule (RAM), and especially a humanised antibody molecule (HAM), having specificity for an antigen present in the T-cell receptor-CD3 complex of most T-cells, to a process for its production using recombinant DNA technology and to its therapeutic use.

In the present application, the term "recombinant antibody molecule" (RAM) is used to describe an antibody produced by an process involving the use of recombinant DNA technology, including any analogues of natural immunoglobulins or their fragments. The term "humanised antibody molecule" (HAM) is used to describe a molecule having an antigen binding site derived from an immunoglobulin from a non-human species, remaining immunoglobulin-derived parts of the molecule being derived from a human immunoglobulin. The antigen binding site may comprise either complete variable domains fused onto constant domains or one or more complementarity determining regions grafted onto appropriate framework regions in the variable domains. The abbreviation "MAb" is used to indicate a monoclonal antibody.

In the description, reference is made to a number of publications by number. The publications are listed in numerical order at the end of the description.

Background of the Invention

Natural immunoglobulins have been known for many years, as have the various fragments thereof, such as the Fab, (Fab')₂ and Fc fragments, which can be derived by enzymatic cleavage. Natural immunoglobulins comprise a generally Y-shaped molecule having an antigen-binding site towards the end of each upper arm. The remainder of the

structure, and particularly the stem of the Y, mediates the effector functions associated with immunoglobulins.

Natural immunoglobulins have been used in assay, diagnosis and, to a more limited extent, therapy. However, such uses, especially in therapy, have been hindered by the polyclonal nature of natural immunoglobulins. A significant step towards the realisation of the potential of immunoglobulins as therapeutic agents was the discovery of techniques for the preparation of monoclonal antibodies of defined specificity (ref. 1). However, most MAbs are produced by fusions of rodent spleen cells with rodent myeloma cells. They are therefore essentially rodent proteins. There are very few reports of the production of human MAbs.

Since most available MAbs are of rodent origin, they are naturally antigenic in humans and thus can give rise to an undesirable immune response termed the HAMA (Human Anti-Mouse Antibody) response. Therefore, the use of rodent MAbs as therapeutic agents in humans is inherently limited by the fact that the human subject will mount an immunological response to the MAb and will either remove it entirely or at least reduce its effectiveness. Thus, in practice, MAbs of rodent origin may not be used in patients for more than one or a few treatments as a HAMA response soon develops rendering the MAb ineffective as well as giving rise to undesirable reactions.

Proposals have therefore been made for making non-human MAbs less antigenic in humans. Such techniques can be generically termed "humanisation" techniques. These techniques generally involve the use of recombinant DNA technology to manipulate DNA sequences encoding the polypeptide chains of the antibody molecule.

Early methods for humanising MAbs involved production of chimeric antibodies in which an antigen binding site comprising the complete variable domains of one antibody is linked to constant domains derived from another antibody. Methods for carrying out such chimerisation procedures are described in EP0120694 (Celltech Limited), EP0125023 (Genentech Inc. and City of Hope), EP-A-0 171496 (Res. Dev. Corp. Japan), EP-A-0 173 494 (Stanford University), and WO 86/01533 (Celltech Limited). This latter Celltech application (WO 86/01533) discloses a process for preparing an antibody molecule having the variable domains from a mouse MAb and the constant domains from a human immunoglobulin. Such humanised chimeric antibodies, however, still contain a significant proportion of non-human amino acid sequence, i.e. the complete non-human variable domains, and thus may still elicit some HAMA response, particularly if administered over a prolonged period [Begent al al Br. J.Cancer, 62: 487 (1990)].

WO 86/01533 also describes the production of an antibody molecule comprising the variable domains of a mouse MAb, the CH1 and CL domains of a human immunoglobulin, and a non-immunoglobulin-derived protein in place of the Fc portion of the human immunoglobulin.

In an alternative approach, described in EP-A-0239400 (Winter), the complementarity determining regions (CDRs) of a mouse MAb have been grafted onto the framework regions of the variable domains of a human immunoglobulin by site directed mutagenesis using long oligonucleotides. There are 3 CDRs (CDR1, CDR2 and CDR3) in each of the heavy and light chain variable regions. Such CDR-grafted humanised antibodies are much less likely to give rise to a HAMA response than humanised chimeric antibodies in view

of the much lower proportion of non-human amino acid sequence which they contain.

The earliest work on humanising MAbs by CDR-grafting was carried out on MAbs recognising synthetic antigens, such as the NP or NIP antigens. However, examples in which a mouse MAb recognising lysozyme and a rat MAb recognising an antigen on human T-cells respectively were humanised by CDR-grafting are shown by Verhoeyen et al (ref. 2) and Riechmann et al (ref. 3). The preparation of CDR-grafted antibody to the antigen on human T cells is also described in WO 89/07452 (Medical Research Council).

In Riechmann et al it was found that transfer of the CDR regions alone (as defined by Kabat refs. 4 and 5) was not sufficient to provide satisfactory antigen binding activity in the CDR-grafted product. Riechmann et al found that it was necessary to convert a serine residue at position 27 of the human sequence to the corresponding rat phenylalanine residue to obtain a CDR-grafted product having satisfactory antigen binding activity. This residue at position 27 of the heavy chain is within the structural loop adjacent to CDR1. A further construct which additionally contained a human serine to rat tyrosine change at position 30 of the heavy chain did not have a significantly altered binding activity over the humanised antibody with the serine to phenylalanine change at position 27 alone. These results indicate that changes to residues of the human sequence outside the CDR regions, in particular in the loop adjacent to CDR1, may be necessary to obtain effective antigen binding activity for CDR-grafted antibodies which recognise more complex antigens. Even so the binding affinity of the best CDR-grafted antibodies obtained was still significantly less than the original MAb.

Very recently Queen et al (ref. 6) have described the preparation of a humanised antibody that binds to the interleukin 2 receptor, by combining the CDRs of a murine MAb (anti-Tac) with human immunoglobulin framework and constant regions. The human framework regions were chosen to maximise homology with the anti-Tac MAb sequence. In addition computer modelling was used to identify framework amino acid residues which were likely to interact with the CDRs or antigen, and mouse amino acids were used at these positions in the humanised antibody.

In WO 90/07861 Queen et al propose four criteria for designing humanised immunoglobulins. The first criterion is to use as the human acceptor the framework from a particular human immunoglobulin that is unusually homologous to the non-human donor immunoglobulin to be humanised, or to use a consensus framework from many human antibodies. The second criterion is to use the donor amino acid rather than the acceptor if the human acceptor residue is unusual and the donor residue is typical for human sequences at a specific residue of the framework. The third criterion is to use the donor framework amino acid residue rather than the acceptor at positions immediately adjacent to the CDRs. The fourth criterion is to use the donor amino acid residue at framework positions at which the amino acid is predicted to have a side chain atom within about 3 Å of the CDRs in a three-dimensional immunoglobulin model and to be capable of interacting with the antigen or with the CDRs of the humanised immunoglobulin. It is proposed that criteria two, three or four may be applied in addition or alternatively to criterion one, and may be applied singly or in any combination.

WO 90/07861 describes in detail the preparation of a single CDR-grafted humanised antibody, a humanised antibody having specificity for the p55 Tac protein of the IL-2 receptor. The combination of all four criteria, as above, were employed in designing this humanised antibody, the variable region frameworks of the human antibody Eu (refs. 4 & 5) being used as acceptor. In the resultant humanised antibody the donor CDRs were as defined by Kabat et al (refs. 4 and 5) and in addition the mouse donor residues were used in place of the human acceptor residues, at positions 27, 30, 48, 66, 67, 89, 91, 94, 103, 104, 105 and 107 in the heavy chain and at positions 48, 60 and 63 in the light chain, of the variable region frameworks. The humanised anti-Tac antibody obtained is reported to have an affinity for p55 of 3×10^9 M^{-1}, about one-third of that of the murine MAb.

OKT3 is a mouse IgG2a/k MAb which recognises an antigen in the T-cell receptor-CD3 complex and has been approved for use in many countries throughout the world as an immunosuppressant in the treatment of acute allograft rejection [Chatenoud et al (ref. 7), and Jeffers et al (ref. 8) However, in view of the murine nature of this MAb, a significant HAMA response, with a major anti-idiotype component, may build up on use. Clearly, it would be highly desirable to diminish or abolish this undesirable HAMA response by suitable humanisation or other recombinant DNA manipulation of this very useful antibody and thus enlarge its area of use. It would also be desirable to apply the techniques of recombinant DNA technology more generally to this useful antibody to prepare RAM products.

Moreover, we have further investigated the preparation of CDR-grafted humanised antibody molecules and have identified a hierarchy of positions within the framework

amino acid identities of the residues are important for obtaining CDR-grafted products with satisfactory binding affinity. This has enabled us to establish a protocol for obtaining satisfactory CDR-grafted products which may be applied very widely irrespective of the level of homology between the donor immunoglobulin and acceptor framework. The set of residues which we have identified as being of critical importance does not coincide with the residues identified by Queen et al (ref. 6).

Summary of the Invention

Accordingly the present invention provides an RAM comprising antigen binding regions derived from the heavy and/or light chain variable regions of a donor anti-CD3 antibody and having anti-CD3 binding specificity, and preferably having an anti-CD3 binding affinity similar to that of OKT3.

Typically the donor anti-CD3 antibody is a rodent MAb.

The RAM of the invention may comprise antigen binding regions from any suitable anti-CD3 antibody, typically a rodent anti-CD3 MAb, e.g. a mouse or rat anti-CD3 MAb. The RAM may comprise a recombinant version of whole or a major part of the amino acid sequence of such a MAb. Also the RAM may comprise only the variable region (VH and/or VL) or one or more CDRs of such a MAb. Especially the RAM may comprise amino acid sequences, whether variable region, CDR or other, derived from the specific anti-CD3 MAb (OKT3) hereinafter specifically described with reference to Figures 1 and 2.

Preferably the RAM of the present invention is a humanised antibody molecule (HAM) having specificity for CD3 and

having an antigen binding site wherein at least one of the complementarity determining regions (CDRs) of the variable domain, usually at least two and preferably all of the CDRs, are derived from a non-human anti-CD3 antibody, e.g. a rodent anti-CD3 MAb.

The RAM may be a chimeric antibody or a CDR-grafted antibody.

Accordingly, in preferred embodiments the invention provides an anti-CD3 CDR-grafted antibody heavy chain having a variable region domain comprising acceptor framework and donor CD3 binding regions wherein the framework comprises donor residues at at least one of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91.

More preferably, the heavy chain framework of the preferred embodiment comprises donor residues at positions 23, 24, 49, 71, 73 and 78 or at positions 23, 24 and 49. The residues at positions 71, 73 and 78 of the heavy chain framework are preferably either all acceptor or all donor residues.

In particularly preferred embodiments the heavy chain framework additionally comprises donor residues at one, some or all of positions 6, 37, 48 and 94. Also it is particularly preferred that residues at positions of the heavy chain framework which are commonly conserved across species, i.e. positions 2, 4, 25, 36, 39, 47, 93, 103, 104, 106 and 107, if not conserved between donor and acceptor, additionally comprise donor residues. Most preferably the heavy chain framework additionally comprises donor residues at positions 2, 4, 6, 25, 36, 37, 39, 47, 48, 93, 94, 103, 104, 106 and 107.

chain framework optionally comprises
donor residues at one, some or all of positions:

1 and 3,

72 and 76,

69 (if 48 is different between donor and acceptor),

38 and 46 (if 48 is the donor residue),

80 and 20 (if 69 is the donor residue),

67,

82 and 18 (if 67 is the donor residue),

91,

88, and

any one or more of 9, 11, 41, 87, 108, 110 and 112.

In the preferred embodiments of the present invention described above and hereinafter, reference is made to CDR-grafted antibody products comprising acceptor framework and donor antigen binding regions. It will be appreciated that the invention is widely applicable to the CDR-grafting of anti-CD3 antibodies in general. Thus, the donor and acceptor antibodies may be anti-CD3 antibodies derived from animals of the same species and even same antibody class or sub-class. More usually, however, the donor and acceptor antibodies are derived from animals of different species. Typically the donor anti-CD3 antibody is a non-human antibody, such as a rodent MAb, and the acceptor antibody is a human antibody.

In the CDR-grafted antibody products of the present invention, the donor CD3 binding region typically comprises at least one CDR from the donor antibody. Usually the donor antigen binding region comprises at least two and preferably all three CDRs of each of the heavy chain and/or light chain variable regions. The CDRs may comprise the Kabat CDRs, the structural loop CDRs or a composite of the Kabat and structural loop CDRs and any combination of any of these. Preferably, the antigen

binding regions of the CDR-grafted heavy chain variable domain comprise CDRs corresponding to the Kabat CDRs at CDR2 (residues 50-65) and CDR3 (residues 95-100) and a composite of the Kabat and structural loop CDRs at CDR1 (residues 26-35).

The residue designations given above and elsewhere in the present application are numbered according to the Kabat numbering (refs. 4 and 5). Thus the residue designations do not always correspond directly with the linear numbering of the amino acid residues. The actual linear amino acid sequence may contain fewer or additional amino acids than in the strict Kabat numbering corresponding to a shortening of, or insertion into, a structural component, whether framework or CDR, of the basic variable domain structure. For example, the heavy chain variable region of the anti-Tac antibody described by Queen et al (ref. 6) contains a single amino acid insert (residue 52a) after residue 52 of CDR2 and a three amino acid insert (residues 82a, 82b and 82c) after framework residue 82, in the Kabat numbering. The correct Kabat numbering of residues may be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a "standard" Kabat numbered sequence.

The invention also provides in a further preferred embodiment a CDR-grafted antibody light chain having a variable region domain comprising acceptor framework and donor CD3 binding regions wherein the framework comprises donor residues at at least one of positions 1 and/or 3 and 46 and/or 47. Preferably the CDR grafted light chain of this preferred embodiment comprises donor residues at positions 46 and/or 47.

The invention also provides in a yet further preferred embodiment a CDR-grafted antibody light chain having a

variable region domain comprising acceptor framework and donor CD3 binding regions wherein the framework comprises donor residues at at least one of positions 46, 48, 58 and 71.

More preferably in this latter embodiment, the framework comprises donor residues at all of positions 46, 48, 58 and 71.

In particularly preferred embodiments of the above preferred embodiments, the light chain framework additionally comprises donor residues at positions 36, 44, 47, 85 and 87. Similarly positions of the light chain framework which are commonly conserved across species, i.e. positions 2, 4, 6, 35, 49, 62, 64-69, 98, 99, 101 and 102, if not conserved between donor and acceptor, additionally comprise donor residues. Most preferably the light chain framework additionally comprises donor residues at positions 2, 4, 6, 35, 36, 38, 44, 47, 49, 62, 64-69, 85, 87, 98, 99, 101 and 102.

In addition the light chain framework of the above preferred embodiments optionally comprises donor residues at one, some or all of positions:
1 and 3,
63,
60 (if 60 and 54 are able to form at potential saltbridge),
70 (if 70 and 24 are able to form a potential saltbridge),
73 and 21 (if 47 is different between donor and acceptor),
37 and 45 (if 47 is different between donor and acceptor), and
any one or more of 10, 12, 40, 80, 103 and 105.

Preferably, the antigen binding regions of the CDR-grafted light chain variable domain comprise CDRs corresponding to the Kabat CDRs at CDR1 (residue 24-34), CDR2 (residues 50-56) and CDR3 (residues 89-97).

The invention further provides in a fourth aspect a CDR-grafted antibody molecule comprising at least one CDR-grafted heavy chain and at least one CDR-grafted light chain as defined above.

The CDR-grafted and humanised antibody molecules and chains of the present invention may comprise: a complete antibody molecule, having full length heavy and light chains; a fragment thereof, such as a Fab, (Fab')$_2$ or FV fragment; a light chain or heavy chain monomer or dimer; or a single chain antibody, e.g. a single chain FV in which heavy and light chain variable regions are joined by a peptide linker; or any other CDR-grafted or humanised antibody product with anti-CD3 binding specificity. Similarly the CDR-grafted heavy and light chain variable region may be combined with other antibody domains as appropriate.

Also the CDR-grafted or humanised heavy or light chains or antibody molecules of the present invention may have attached to them an effector or reporter molecule. For instance, it may have a macrocycle, for chelating a heavy metal atom, or a toxin, such as ricin, attached to it by a covalent bridging structure. Alternatively, the procedures of recombinant DNA technology may be used to produce an immunoglobulin molecule in which the Fc fragment or CH3 domain of a complete immunoglobulin molecule has been replaced by, or has attached thereto by peptide linkage, a functional non-immunoglobulin protein, such as an enzyme or toxin molecule.

g grafted antibody products, any appropriate
acceptor variable region framework sequences may be used
having regard to class/type of the donor antibody from
which the antigen binding regions are derived.
Preferably, the type of acceptor framework used is of the
same/similar class/type as the donor antibody.
Conveniently, the framework may be chosen to maximise/
optimise homology with the donor antibody sequence
particularly at positions close or adjacent to the CDRs.
However, a high level of homology between donor and
acceptor sequences is not important for application of the
present invention.   The present invention identifies a
hierarchy of framework residue positions at which donor
residues may be important or desirable for obtaining a
CDR-grafted antibody product having satisfactory binding
properties.   The present invention advantageously enables
the preparation of CDR-grafted antibody products having
binding affinities similar to, and even in some cases
better than the corresponding donor antibody product, e.g.
OKT3 product.   Preferably, the CDR-grafted antibody
products of the invention have binding affinities of at
least about 10^5 M^{-1}, preferably at least about 10^8 M^{-1} and
especially within the range 10^8-10^{12} M^{-1}.   In principle,
the present invention is applicable to any combination of
donor and acceptor antibodies irrespective of the level of
homology between their sequences.   A protocol for
applying the invention to any particular donor-acceptor
antibody pair is given hereinafter.   Examples of human
frameworks which may be used are KOL, NEWM, REI, EU, LAY
and POM (refs. 4 and 5);   for instance KOL and NEWM for
the heavy chain and REI for the light chain and EU, LAY
and POM for both the heavy chain and the light chain.

Also the constant region domains of the products of the
invention may be selected having regard to the proposed
function of the antibody in particular the effector

functions which may be required. For example, the constant region domains may be human IgA, IgE, IgG or IgM domains. In particular, IgG human constant region domains may be used, especially of the IgG1 and IgG3 isotypes, when the humanised antibody molecule is intended for therapeutic uses, and antibody effector functions are required. Alternatively, IgG2 and IgG4 isotypes may be used when the humanised antibody molecule is intended for therapeutic purposes and antibody effector functions are not required, e.g. for simple blocking of the T-cell receptor-CD3 complex.

However, the remainder of the antibody molecules need not comprise only protein sequences from immunoglobulins. For instance, a gene may be constructed in which a DNA sequence encoding part of a human immunoglobulin chain is fused to a DNA sequence encoding the amino acid sequence of a polypeptide effector or reporter molecule.

Preferably the CDR-grafted heavy and light chain and antibody molecule products are produced by recombinant DNA technology.

Thus in further aspects the invention also includes DNA sequences coding for the RAMs, HAMs and CDR-grafted heavy and light chains, cloning and expression vectors containing the DNA sequences, host cells transformed with the DNA sequences and processes for producing the CDR-grafted antibody molecules comprising expressing the DNA sequences in the transformed host cells.

The general methods by which the vectors may be constructed, transfection methods and culture methods are well known per se and form no part of the invention. Such methods are shown, for instance, in references 9 and 10.

The DNA sequences which encode the anti-CD3 donor amino acid sequence may be obtained by methods well known in the art.   For example the anti-CD3 coding sequences may be obtained by genomic cloning, or cDNA cloning from suitable hybridoma cell lines, e.g. the OKT3 cell line hereinafter specifically described.   Positive clones may be screened using appropriate probes for the heavy and light chain genes in question.   Also PCR cloning may be used.

DNA coding for acceptor, e.g. human acceptor, sequences may be obtained in any appropriate way.   For example DNA sequences coding for preferred human acceptor frameworks such as KOL, REI, EU and NEWM, are widely available to workers in the art.

The standard techniques of molecular biology may be used to prepare DNA sequences coding for the chimeric and CDR-grafted products.   Desired DNA sequences may be synthesised completely or in part using oligonucleotide synthesis techniques.   Site-directed mutagenesis and polymerase chain reaction (PCR) techniques may be used as appropriate.   For example oligonucleotide directed synthesis as described by Jones et al (ref. 17) may be used.   Also oligonucleotide directed mutagenesis of a pre-exising variable region as, for example, described by Verhoeyen et al (ref. 2) or Riechmann et al (ref. 3) may be used.   Also enzymatic filling in of gapped oligonucleotides using T4 DNA polymerase as, for example, described by Queen et al (ref. 6) may be used.

Any suitable host cell/vector system may be used for expression of the DNA sequences coding for the CDR-grafted heavy and light chains.   Bacterial e.g. E. coli, and other microbial systems may be used, in particular for expression of antibody fragments such as FAb and (Fab')$_2$ fragments, and especially FV fragments and single chain

antibody fragments e.g. single chain FVs. Eucaryotic,
e.g. mammalian, host cell expression systems may be used,
in particular, for production of larger CDR-grafted
antibody products, including complete antibody
molecules. Suitable mammalian host cells include CHO
cells and myeloma or hybridoma cell lines.

Thus, according to a further aspect the present invention
provides a process for producing an anti-CD3 RAM which
process comprises:

(a) producing in an expression vector an operon having a
DNA sequence which encodes an antibody heavy chain
wherein at least one CDR of the variable domain is
derived from a donor anti-CD3 antibody and remaining
immunglobulin-derived parts of the antibody chain are
derived from an acceptor immunoglobulin;

and/or

(b) producing in an expression vector an operon having a
DNA sequence which encodes a complementary antibody
light chain wherein at least one CDR of the variable
domain is derived from a donor anti-CD3 antibody and
the remaining immunoglobulin-derived parts of the
antibody chain are derived from an acceptor
immunoglobulin;

(c) transfecting a host cell with the or each vector;
and

(d) culturing the transfected cell line to produce the
RAM.

The RAM may comprise only heavy or light chain-derived
polypeptide, in which case only a heavy chain or light
chain polypeptide coding sequence is used to transfect the
host cells.

For production of RAMs comprising both heavy and light chains, the cell line may be transfected with two vectors. The first vector may contain an operon encoding a light chain-derived polypeptide and the second vector may contain an operon encoding a heavy chain-derived polypeptide. Preferably, the vectors are identical except in so far as the coding sequences and selectable markers are concerned so as to ensure as far as possible that each polypeptide chain is equally expressed. Alternatively, a single vector may be used, the vector including the sequences encoding both light chain- and heavy chain-derived polypeptides.

The DNA in the coding sequences for the light and heavy chains may comprise cDNA or genomic DNA or both. However, it is preferred that the DNA sequence encoding the heavy or light chain comprises, at least partially, genomic DNA. Most preferably, the heavy or light chain encoding sequence comprises a fusion of cDNA and genomic DNA.

The present invention also includes therapeutic and diagnostic compositions comprising the RAMs, HAMs and CDR-grafted light and heavy chains and molecules of the invention and uses of such compositions in therapy and diagnosis.

Accordingly in a further aspect the invention provides a therapeutic or diagnostic composition comprising a RAM, HAM or CDR-grafted antibody heavy or light chain or molecule according to previous aspects of the invention in combination with a pharmaceutically acceptable carrier, diluent or excipient.

Accordingly also the invention provides a method of therapy or diagnosis comprising administering an effective

amount of a RAM, HAM or CDR-grafted antibody heavy or
light chain or molecule according to previous aspects of
the invention to a human or animal subject.

The RAM, HAM and CDR-grafted products of the present
invention may be used for any of the therapeutic uses for
which anti CD3 antibodies, e.g. OKT3, have been used or
may be used in the future. For example, the products may
be used as ummunosuppressants, e.g. in the treatment of
acute allograft rejection.

A preferred protocol for obtaining CDR-grafted antibody
heavy and light chains in accordance with the present
invention is set out below together with the rationale by
which we have derived this protocol. This protocol and
rationale are given without prejudice to the generality of
the invention as hereinbefore described and defined.

Protocol
It is first of all necessary to sequence the DNA coding
for the heavy and light chain variable regions of the
donor antibody, to determine their amino acid sequences.
It is also necessary to choose appropriate acceptor heavy
and light chain variable regions, of known amino acid
sequence. The CDR-grafted chain is then designed
starting from the basis of the acceptor sequence. It
will be appreciated that in some cases the donor and
acceptor amino acid residues may be identical at a
particular position and thus no change of acceptor
framework residue is required.

1. As a first step donor residues are substituted for
    acceptor residues in the CDRs. For this purpose the
    CDRs are preferably defined as follows:

Heavy chain - CDR1:   residues 26-35

                - CDR2:   residues 50-65

                - CDR3:   residues 95-102

Light chain - CDR1:   residues 24-34

                - CDR2:   residues 50-56

                - CDR3:   residues 89-97

The positions at which donor residues are to be substituted for acceptor in the framework are then chosen as follows, first of all with respect to the heavy chain and subsequently with respect to the light chain.

2.   Heavy Chain

2.1 Choose donor residues at all of positions 23, 24, 49, 71, 73 and 78 of the heavy chain or all of positions 23, 24 and 49 (71, 73 and 78 are either all donor or all acceptor).

2.2 Check that the following have the same amino acid in donor and acceptor sequences, and if not preferably choose the donor: 2, 4, 6, 25, 36, 37, 39, 47, 48, 93, 94, 103, 104, 106 and 107.

2.3 To further optimise affinity consider choosing donor residues at one, some or any of:

    i.   1, 3

    ii.   72, 76

    iii. If 48 is different between donor and acceptor sequences, consider 69

    iv. If at 48 the donor residue is chosen, consider 38 and 46

    v.   If at 69 the donor residue is chosen, consider 80 and then 20

    vi. 67

vii. If at 67 the donor residue is chosen, consider 82 and then 18

viii. 91

ix. 88

x.   9, 11, 41, 87, 108, 110, 112

3. **Light Chain**

3.1 Choose donor at 46, 48, 58 and 71

3.2 Check that the following have the same amino acid in donor and acceptor sequences, if not preferably choose donor:

2, 4, 6, 35, 38, 44, 47, 49, 62, 64-69 inclusive, 85, 87, 98, 99, 101 and 102

3.3 To further optimise affinity consider choosing donor residues at one, some or any of:

i.   1, 3

ii. 63

iii. 60, if 60 and 54 are able to form a potential saltbridge

iv. 70, if 70 and 24 are able to form a potential saltbridge

v.   73 and 21, if 47 is different between donor and acceptor

vi. 37 and 45, if 47 is different between donor and acceptor

vii. 10, 12, 40, 80, 103, 105

Rationale

In order to transfer the binding site of an antibody into a different acceptor framework, a number of factors need to be considered.

1.

<u>The extent of the CDRs</u>

The CDRs (Complementary Determining Regions) were defined by Wu and Kabat (refs. 4 and 5) on the basis of an analysis of the variability of different regions of antibody variable regions. Three regions per domain were recognised. In the light chain the sequences are 24-34, 50-56, 89-97 (numbering according to Kabat (ref. 4), Eu Index) inclusive and in the heavy chain the sequences are 31-35, 50-65 and 95-102 inclusive.

When antibody structures became available it became apparent that these CDR regions corresponded in the main to loop regions which extended from the β barrel framework of the light and heavy variable domains. For H1 there was a discrepancy in that the loop was from 26 to 32 inclusive and for H2 the loop was 52-56 and for L2 from 50 to 53. However, with the exception of H1 the CDR regions encompassed the loop regions and extended into the β strand frameworks. In H1 residue 26 tends to be a serine and 27 a phenylalanine or tyrosine, residue 29 is a phenylalanine in most cases. Residues 28 and 30 which are surface residues exposed to solvent might be involved in antigen-binding. A prudent definition of the H1 CDR therefore would include residues 26-35 to include both the loop region and the hypervariable residues 33-35.

It is of interest to note the example of Riechmann <u>et al</u> (ref. 3), who used the residue 31-35 choice for CDR-H1. In order to produce efficient antigen binding residue 27 also needed to be recruited from the donor (rat) antibody.

2.   Non-CDR residues which contribute to antigen binding

By examination of available X-ray structures we have identified a number of residues which may have an effect on net antigen binding and which can be demonstrated by experiment. These residues can be sub-divided into a number of groups.

2.1   Surface residues near CDR [all numbering as in Kabat et al (ref. 4)].

2.1.1. Heavy Chain - Key residues are 23, 71 and 73. Other residues which may contribute to a lesser extent are 1, 3 and 76. Finally 25 is usually conserved but the murine residue should be used if there is a difference.

2.1.2   Light Chain - Many residues close to the CDRs, e.g. 63, 65, 67 and 69 are conserved. If conserved none of the surface residues in the light chain are likely to have a major effect. However, if the murine residue at these positions is unusual, then it would be of benefit to analyse the likely contribution more closely. Other residues which may also contribute to binding are 1 and 3, and also 60 and 70 if the residues at these positions and at 54 and 24 respectively are potentially able to form a salt bridge i.e. 60 + 54; 70 + 24.

2.2   Packing residues near the CDRs.

2.2.1. Heavy Chain - Key residues are 24, 49 and 78. Other key residues would be 36 if not a tryptophan, 94 if not an arginine, 104 and 106 if not glycines and 107 if not a threonine. Residues which may make a further contribution to stable packing of the heavy chain and hence improved affinity are 2, 4, 6, 38, 46, 67 and 69. 67 packs against the CDR residue 63 and

this pair could be either both mouse or both human. Finally, residues which contribute to packing in this region but from a longer range are 18, 20, 80, 82 and 86. 82 packs against 67 and in turn 18 packs against 82. 80 packs against 69 and in turn 20 packs against 80. 86 forms an H bond network with 38 and 46. Many of the mouse-human differences appear minor e.g. Leu-Ile, but could have an minor impact on correct packing which could translate into altered positioning of the CDRs.

2.2.2. Light Chain - Key residues are 48, 58 and 71. Other key residues would be 6 if not glutamine, 35 if not tryptophan, 62 if not phenylalanine or tryosine, 64, 66, 68, 99 and 101 if not glycines and 102 if not a threonine. Residues which make a further contribution are 2, 4, 37, 45 and 47. Finally residues 73 and 21 and 19 may make long distance packing contributions of a minor nature.

2.3. Residues at the variable domain interface between heavy and light chains - In both the light and heavy chains most of the non-CDR interface residues are conserved. If a conserved residue is replaced by a residue of different character, e.g. size or charge, it should be considered for retention as the murine residue.

2.3.1. Heavy Chain - Residues which need to be considered are 37 if the residue is not a valine but is of larger side chain volume or has a charge or polarity. Other residues are 39 if not a glutamine, 45 if not a leucine, 47 if not a tryptophan, 91 if not a phenylalanine or tyrosine, 93 if not an alanine and 103 if not a tryptophan. Residue 89 is also at the interface

but is not in a position where the side chain could be of great impact.

2.3.2. Light Chain - Residues which need to be considered are 36, if not a tyrosine, 38 if not a glutamine, 44 if not a proline, 46, 49 if not a tyrosine, residue 85, residue 87 if not a tyrosine and 98 if not a phenylalanine.

2.4. Variable-Constant region interface - The elbow angle between variable and constant regions may be affected by alterations in packing of key residues in the variable region against the constant region which may affect the position of V_L and V_H with respect to one another. Therefore it is worth noting the residues likely to be in contact with the constant region. In the heavy chain the surface residues potentially in contact with the variable region are conserved between mouse and human antibodies therefore the variable region contact residues may influence the V-C interaction. In the light chain the amino acids found at a number of the constant region contact points vary, and the V & C regions are not in such close proximity as the heavy chain. Therefore the influences of the light chain V-C interface may be minor.

2.4.1. Heavy Chain - Contact residues are 7, 11, 41, 87, 108, 110, 112.

2.4.2. Light Chain - In the light chain potentially contacting residues are 10, 12, 40, 80, 83, 103 and 105.

The above analysis coupled with our considerable practical experimental experience in the CDR-grafting of a number of different antibodies have lead us to the protocol given above.

The present invention is now described, by way of example only, with reference to the accompanying Figures 1 - 13.

Brief Description of the Figures

Figure 1   shows DNA and amino acid sequences of the OKT3 light chain;

Figure 2   shows DNA and amino acid sequences of the OKT3 heavy chain;

Figure 3   shows the alignment of the OKT3 light variable region amino acid sequence with that of the light variable region of the human antibody REI;

Figure 4   shows the alignment of the OKT3 heavy variable region amino acid sequence with that of the heavy variable region of the human antibody KOL;

Figure 5   shows the heavy variable region amino acid sequences of OKT3, KOL and various corresponding CDR grafts;

Figure 6   shows the light variable region amino acid sequences of OKT3, REI and various corresponding CDR grafts;

Figure 7   shows a graph of binding assay results for various grafted OKT3 antibodies'

Figure 8   shows a graph of blocking assay results for various grafted OKT3 antibodies;

Figure 9   shows a similar graph of blocking assay results;

Figure 10   shows similar graphs for both binding assay and blocking assay results;

Figure 11   shows further similar graphs for both binding assay and blocking assay results;

Figure 12   shows a graph of competition assay results for a minimally grafted OKT3 antibody compared with the OKT3 murine reference standard, and

Figure 13   shows a similar graph of competition assay results comparing a fully grafted OKT3 antibody with the murine reference standard.

DETAILED DESCRIPTION OF EMBODIMENTS OF THE INVENTION

EXAMPLE

MATERIAL AND METHODS

1. ### INCOMING CELLS
    Hybridoma cells producing antibody OKT3 were
    provided by Ortho (seedlot 4882.1) and were grown
    up in antibiotic free Dulbecco's Modified Eagles
    Medium (DMEM) supplemented with glutamine and 5%
    foetal calf serum, and divided to provide both an
    overgrown supernatant for evaluation and cells
    for extraction of RNA. The overgrown
    supernatant was shown to contain 250 ug/mL murine
    IgG2a/kappa antibody. The supernatant was
    negative for murine lambda light chain and IgG1,
    IgG2b, IgG3, IgA and IgM heavy chain. 20mL of
    supernatant was assayed to confirm that the
    antibody present was OKT3.

2. ### MOLECULAR BIOLOGY PROCEDURES
    Basic molecular biology procedures were as
    described in Maniatis et al (ref. 9) with, in
    some cases, minor modifications. DNA sequencing
    was performed as described in Sanger et al (ref.
    11) and the Amersham International Plc sequencing
    handbook. Site directed mutagenesis was as
    described in Kramer et al (ref. 12) and the
    Anglian Biotechnology Ltd. handbook. COS cell
    expression and metabolic labelling studies were
    as described in Whittle et al (ref. 13)

3.   RESEARCH ASSAYS

3.1.   ASSEMBLY ASSAYS

Assembly assays were performed on supernatants from transfected COS cells to determine the amount of intact IgG present.

3.1.1.   COS CELLS TRANSFECTED WITH MOUSE OKT3 GENES

The assembly assay for intact mouse IgG in COS cell supernatants was an ELISA with the following format:

96 well microtitre plates were coated with F(ab')$_2$ goat anti-mouse IgG Fc. The plates were washed in water and samples added for 1 hour at room temperature. The plates were washed and F(ab')$_2$ goat anti-mouse IgG F(ab')$_2$ (HRPO conjugated) was then added. Substrate was added to reveal the reaction. UPC10, a mouse IgG2a myeloma, was used as a standard.

3.1.2.   COS AND CHO CELLS TRANSFECTED WITH CHIMERIC OR CDR-GRAFTED OKT3 GENES

The assembly assay for chimeric or CDR-grafted antibody in COS cell supernatants was an ELISA with the following format:

96 well microtitre plates were coated with F(ab')$_2$ goat anti-human IgG Fc. The plates were washed and samples added and incubated for 1 hour at room temperature. The plates were washed and monoclonal mouse anti-human kappa chain was added for 1 hour at room temperature.

The plates were washed and F(ab')$_2$ goat anti-mouse IgG Fc (HRPO conjugated) was added. Enzyme substrate was added to reveal the reaction. Chimeric B72.3 (IgG4) (ref. 13) was used as a standard. The use of a monoclonal anti-kappa chain in this assay allows grafted antibodies to be read from the chimeric standard.

3.2.   ASSAY FOR ANTIGEN BINDING ACTIVITY

Material from COS cell supernatants was assayed for OKT3 antigen binding activity onto CD3 positive cells in a direct assay.   The procedure was as follows:

HUT 78 cells (human T cell line, CD3 positive) were maintained in culture.   Monolayers of HUT 78 cells were prepared onto 96 well ELISA plates using poly-L-lysine and glutaraldehyde.

Samples were added to the monolayers for 1 hour at room temperature.

The plates were washed gently using PBS. $F(ab')_2$ goat anti-human IgG Fc (HRPO conjugated) or $F(ab')_2$ goat anti-mouse IgG Fc (HRPO conjugated) was added as appropriate for humanised or mouse samples.   Substrate was added to reveal the reaction.

The negative control for the cell-based assay was chimeric B72.3.   The positive control was mouse Orthomune OKT3 or chimeric OKT3, when available.   This cell-based assay was difficult to perform, and an alternative assay was developed for CDR-grafted OKT3 which was more sensitive and easier to carry out.

In this system, CDR-grafted OKT3 produced by COS cells was tested for its ability to bind to the CD3-positive HPB-ALL (human peripheral blood acute lymphocytic leukemia) cell line.   It was also tested for its ability to block the binding of murine OKT3 to these cells.   Binding was measured by the following procedure: HPB-ALL cells were harvested from tissue culture.   Cells were incubated at 4°C for 1 hour with various dilutions of test antibody, positive control antibody, or negative control antibody.   The

cells were washed once and incubated at 4°C for 1 hour with an FITC-labelled goat anti-human IgG (Fc- specific, mouse absorbed). The cells were washed twice and analysed by cytofluorography. Chimeric OKT3 was used as a positive control for direct binding. Cells incubated with mock-transfected COS cell supernatant, followed by the FITC-labelled

goat anti-human IgG, provided the negative control.

To test the ability of CDR-grafted OKT3 to block murine OKT3 binding, the HPB-ALL cells were incubated at 4°C for 1 hour with various dilutions of test antibody or control antibody. A fixed saturating amount of FITC OKT3 was added. The samples were incubated for 1 hour at 4°C, washed twice and analysed by cytofluorography. FITC-labelled OKT3 was used as a positive control to determine maximum binding. Unlabelled murine OKT3 served as a reference standard for blocking. Negative controls were unstained cells with or without mock-transfected cell supernatant.

The ability of the CDR-grafted OKT3 light chain to bind CD3-positive cells and block the binding of murine OKT3 was initially tested in combination with the chimeric OKT3 heavy chain. The chimeric OKT3 heavy chain is composed of the murine OKT3 variable region and the human IgG4 constant region. The chimeric heavy chain gene is expressed in the same expression vector used for the CDR-grafted genes. The CDR-grafted light chain expression vector and the chimeric heavy chain expression vector were co-transfected into COS cells. The fully chimeric OKT3 antibody (chimeric light chain and chimeric heavy

chain) was found to be fully capable of binding to CD3 positive cells and blocking the binding of murine OKT3 to these cells.

3.3 DETERMINATION OF RELATIVE BINDING AFFINITY

The relative binding affinities of CDR-grafted anti-CD3 monoclonal antibodies were determined by competition binding (ref. 6) using the HPB-ALL human T cell line as a source of CD3 antigen, and fluorescein-conjugated murine OKT3 (Fl-OKT3) of known binding affinity as a tracer antibody.

The binding affinity of Fl-OKT3 tracer antibody was determined by a direct binding assay in which increasing amounts of Fl-OKT3 were incubated with HPB-ALL (5×10^5) in PBS with 5% foetal calf serum for 60 min. at 4°C. Cells were washed, and the fluorescence intensity was determined on a FACScan flow cytometer calibrated with quantitative microbead standards (Flow Cytometry Standards, Research Triangle Park, NC). Fluorescence intensity per antibody molecule (F/P ratio) was determined by using microbeads which have a predetermined number of mouse IgG antibody binding sites (Simply Cellular beads, Flow Cytometry Standards). F/P equals the fluorescence intensity of beads saturated with Fl-OKT3 divided by the number of binding sites per bead. The amount of bound and free Fl-OKT3 was calculated from the mean fluorescence intensity per cell, and the ratio of bound/free was plotted against the number of moles of antibody bound. A linear fit was used to determine the affinity of binding (absolute value of the slope).

For competitive binding, increasing amounts of competitor antibody were added to a sub-saturating dose of Fl-OKT3 and incubated with

5x10^5 HPB-ALL in 200 μl of PBS with 5% foetal
calf serum, for 60 min at 4°C. The fluorescence
intensities of the cells were measured on a
FACScan flow cytometer calibrated with
quantitative microbead standards.
The concentrations of bound and free Fl-OKT3 were
calculated. The affinities of competing anti-
bodies were calculated from the equation
[X]-[OKT3] = (1/Kx) - (1/Ka), where Ka is the
affinity of murine OKT3, Kx is the affinity of
competitor X, [ ] is the concentration of
competitor antibody at which bound/free binding
is R/2, and R is the maximal bound/free binding.

4.   cDNA LIBRARY CONSTRUCTION

4.1.   mRNA PREPARATION AND cDNA SYNTHESIS
OKT3 producing cells were grown as described
above and 1.2 x 10^9 cells harvested and mRNA
extracted using the guanidinium/LiCl extraction
procedure. cDNA was prepared by priming from
Oligo-dT to generate full length cDNA. The cDNA
was methylated and EcoR1 linkers added for
cloning.

4.2.   LIBRARY CONSTRUCTION
The cDNA library was ligated to pSP65 vector DNA
which had been EcoR1 cut and the 5′ phosphate
groups removed by calf intestinal phosphatase
(EcoR1/CIP). The ligation was used to transform
high transformation efficiency Escherichia coli
(E.coli) HB101. A cDNA library was prepared.
3600 colonies were screened for the light chain
and 10000 colonies were screened for the heavy
chain.

5. SCREENING

E.coli colonies positive for either heavy or light chain probes were identified by oligonucleotide screening using the oligonucleotides:

5' TCCAGATGTTAACTGCTCAC for the light chain, which is complementary to a sequence in the mouse kappa constant region, and 5' CAGGGGCCAGTGGATGGATAGAC for the heavy chain which is complementary to a sequence in the mouse IgG2a constant CH1 domain region. 12 light chain and 9 heavy chain clones were identified and taken for second round screening. Positive clones from the second round of screening were grown up and DNA prepared. The sizes of the gene inserts were estimated by gel electrophoresis and inserts of a size capable of containing a full length cDNA were subcloned into M13 for DNA sequencing.

6. DNA SEQUENCING

Clones representing four size classes for both heavy and light chains were obtained in M13. DNA sequence for the 5' untranslated regions, signal sequences, variable regions and 3' untranslated regions of full length cDNAs [Figures 1(a) and 2(a)] were obtained and the corresponding amino acid sequences predicted [(Figures 1(b) and 2(b)]. In Figure 1(a) the untranslated DNA regions are shown in uppercase, and in both Figures 1 and 2 the signal sequences are underlined.

7. CONSTRUCTION OF cDNA EXPRESSION VECTORS

Celltech expression vectors are based on the plasmid pEE6hCMV (ref. 14). A polylinker for the insertion of genes to be expressed has been

introduced after the major immediate early
promoter/enhancer of the human Cytomegalovirus
(hCMV).   Marker genes for selection of the
plasmid in transfected eukaryotic cells can be
inserted as BamH1 cassettes in the unique BamH1
site of pEE6 hCMV; for instance, the neo marker
to provide pEE6 hCMV neo.   It is usual practice
to insert the neo and gpt markers prior to
insertion of the gene of interest, whereas the GS
marker is inserted last because of the presence
of internal EcoR1 sites in the cassette.
The selectable markers are expressed from the
SV40 late promoter which also provides an origin
of replication so that the vectors can be used
for expression in the COS cell transient
expression system.
The mouse sequences were excised from the M13
based vectors described above as EcoR1 fragments
and cloned into either pEE6-hCMV-neo for the
heavy chain and into EE6-hCMV-gpt for the light
chain to yield vectors pJA136 and pJA135
respectively.

8.
EXPRESSION OF cDNAS IN COS CELLS
Plasmids pJA135 and pJA136 were co-transfected
into COS cells and supernatant from the transient
expression experiment was shown to contain
assembled antibody which bound to T-cell enriched
lymphocytes.   Metabolic labelling experiments
using ^{35}S methionine showed expression and
assembly of heavy and light chains.

9.
CONSTRUCTION OF CHIMERIC GENES
Construction of chimeric genes followed a
previously described strategy [Whittle et al
(ref. 13)].   A restriction site near the 3′ end

of the variable domain sequence is identified and
used to attach an oligonucleotide adapter coding
for the remainder of the mouse variable region
and a suitable restriction site for attachment to
the constant region of choice.

9.1.   LIGHT CHAIN GENE CONSTRUCTION

The mouse light chain cDNA sequence contains an
AvaI site near the 3' end of the variable region
[Fig. 1(a)].   The majority of the sequence of
the variable region was isolated as a 396 bp.
EcoR1-AvaI fragment.   An oligonucleotide adapter
was designed to replace the remainder of the 3'
region of the variable region from the AvaI site
and to include the 5' residues of the human
constant region up to and including a unique NarI
site which had been previously engineered into
the constant region.

A HindIII site was introduced to act as a marker
for insertion of the linker.

The linker was ligated to the V_L fragment and the
413 bp EcoR1-NarI adapted fragment was purified
from the ligation mixture.

The constant region was isolated as an NarI-BamHI
fragment from an M13 clone NW361 and was ligated
with the variable region DNA into an
EcoR1/BamH1/ClP pSP65 treated vector in a three
way reaction to yield plasmid JA143.   Clones
were isolated after transformation into E.coli
and the linker and junction sequences were
confirmed by the presence of the HindIII site and
by DNA sequencing.

9.2   LIGHT CHAIN GENE CONSTRUCTION - VERSION 2

The construction of the first chimeric light
chain gene produces a fusion of mouse and human
amino acid sequences at the variable-constant
region junction.   In the case of the OKT3 light

.......Leu Glu-Ile-<u>Asn-Arg</u>/   -/<u>Thr</u>-Val-Ala   -Ala
                     VARIABLE   CONSTANT

This arrangement of sequence introduces a potential site for Asparagine (Asn) linked (N-linked) glycosylation at the V-C junction. Therefore, a second version of the chimeric light chain oligonucleotide adapter was designed in which the threonine (Thr), the first amino acid of the human constant region, was replaced with the equivalent amino acid from the mouse constant region, Alanine (Ala).

An internal Hind111 site was not included in this adapter, to differentiate the two chimeric light chain genes.

The variable region fragment was isolated as a 376 bp EcoR1-Aval fragment.   The oligonucleotide linker was ligated to Nar1 cut pNW361 and then the adapted 396bp constant region was isolated after recutting the modified pNW361 with EcoR1. The variable region fragment and the modified constant region fragment were ligated directly into EcoR1/C1P treated pEE6hCMVneo to yield pJA137.

Initially all clones examined had the insert in the incorrect orientation.   Therefore, the insert was re-isolated and recloned to turn the insert round and yield plasmid pJA141.   Several clones with the insert in the correct orientation were obtained and the adapter sequence of one was confirmed by DNA sequencing

9.3.   HEAVY CHAIN GENE CONSTRUCTION
9.3.1.   CHOICE OF HEAVY CHAIN GENE ISOTYPE
The constant region isotype chosen for the heavy chain was human IgG4.

9.3.2.   GENE CONSTRUCTION

The heavy chain cDNA sequence showed a Ban1 site
near the 3' end of the variable region [Fig.
2(a)].

The majority of the sequence of the variable
region was isolated as a 426bp. EcoR1/ClP/Ban1
fragment.   An oligonucleotide adapter was
designated to replace the remainder of the 3'
region of the variable region from the Ban1 site
up to and including a unique HindIII site which
had been previously engineered into the first two
amino acids of the constant region.

The linker was ligated to the V_H fragment and the
EcoR1-Hindlll adapted fragment was purified from
the ligation mixture.

The variable region was ligated to the constant
region by cutting pJA91 (ref. ??) with EcoR1 and
Hindlll removing the intron fragment and
replacing it with the V_H to yield pJA142.

Clones were isolated after transformation into
E.coli JM101 and the linker and junction
sequences were confirmed by DNA sequencing.
(N.B. The Hindlll site is lost on cloning).

10.   CONSTRUCTION OF CHIMERIC EXPRESSION VECTORS

10.1.   neo AND gpt VECTORS

The chimeric light chain (version 1) was removed
from pJA143 as an EcoR1 fragment and cloned into
EcoR1/ClP treated pEE6hCMVneo expression vector
to yield pJA145.   Clones with the insert in the
correct orientation were identified by
restriction mapping.

The chimeric light chain (version 2) was
constructed as described above.

The chimeric heavy chain gene was isolated from
pJA142 as a 2.5Kbp EcoR1/BamH1 fragment and

cloned into the EcoR1/Bcl1/C1P treated vector fragment of a derivative of pEE6hCMVgpt to yield plasmid pJA144.

10.2.   GS SEPARATE VECTORS

GS versions of pJA141 and pJA144 were constructed by replacing the neo and gpt cassettes by a BamH1/Sal1/C1P treatment of the plasmids, isolation of the vector fragment and ligation to a GS-containing fragment from the plasmid pRO49 to yield the light chain vector pJA179 and the heavy chain vector pJA180.

10.3.   GS SINGLE VECTOR CONSTRUCTION

Single vector constructions containing the cL (chimeric light), cH (chimeric heavy) and GS genes on one plasmid in the order cL-cH-GS, or cH-cL-GS and with transcription of the genes being head to tail e.g. cL>cH>GS were constructed.   These plasmids were made by treating pJA179 or pJA180 with BamH1/C1P and ligating in a Bgl11/Hind111 hCMV promoter cassette along with either the Hind111/BamH1 fragment from pJA141 into pJA180 to give the cH-cL-GS plasmid pJA182 or the Hind111/BamH1 fragment from pJA144 into pJA179 to give the cL-cH-GS plasmid pJA181.

11.   EXPRESSION OF CHIMERIC GENES
11.1.   EXPRESSION IN COS CELLS

The chimeric antibody plasmid pJA145 (cL) and pJA144 (cH) were co-transfected into COS cells and supernatant from the transient expression experiment was shown to contain assembled antibody which bound to the HUT 78 human T-cell line.   Metabolic labelling experiments using ^{35} methionine showed expression and assembly of heavy and light chains.   However the light chain

mobility seen on reduced gels suggested that the potential glycosylation site was being glycosylated. Expression in COS cells in the presence of tunicamycin showed a reduction in size of the light chain to that shown for control chimeric antibodies and the OKT3 mouse light chain. Therefore JA141 was constructed and expressed. In this case the light chain did not show an aberrant mobility or a size shift in the presence or absence of tunicamycin. This second version of the chimeric light chain, when expressed in association with chimeric heavy (cH) chain, produced antibody which showed good binding to HUT 78 cells. In both cases antigen binding was equivalent to that of the mouse antibody.

11.2 EXPRESSION IN CHINESE HAMSTER OVARY (CHO) CELLS
Stable cell lines have been prepared from plasmids PJA141/pJA144 and from pJA179/pJA180, pJA181 and pJA182 by transfection into CHO cells.

12. CDR-GRAFTING
The approach taken was to try to introduce sufficient mouse residues into a human variable region framework to generate antigen binding activity comparable to the mouse and chimeric antibodies.

12.1. VARIABLE REGION ANALYSIS
From an examination of a small database of structures of antibodies and antigen-antibody complexes it is clear that only a small number of antibody residues make direct contact with antigen. Other residues may contribute to antigen binding by positioning the contact residues in favourable configurations and also by inducing a stable packing of the individual

variable domains and stable interaction of the light and heavy chain variable domains. The residues chosen for transfer can be identified in a number of ways:

(a)   By examination of antibody X-ray crystal structures the antigen binding surface can be predominantly located on a series of loops, three per domain, which extend from the B-barrel framework.

(b)   By analysis of antibody variable domain sequences regions of hypervariability [termed the Complementarity Determining Regions (CDRs) by Wu and Kabat (ref. 5)] can be identified.   In the most but not all cases these CDRs correspond to, but extend a short way beyond, the loop regions noted above.

(c)   Residues not identified by (a) and (b) may contribute to antigen binding directly or indirectly by affecting antigen binding site topology, or by inducing a stable packing of the individual variable domains and stabilising the inter-variable domain interaction.   These residues may be identified either by superimposing the sequences for a given antibody on a known structure and looking at key residues for their contribution, or by sequence alignment analysis and noting "idiosyncratic" residues followed by examination of their structural location and likely effects.

12.1.1.   LIGHT CHAIN

Figure 3 shows an alignment of sequences for the human framework region RE1 and the OKT3 light variable region.   The structural loops (LOOP) and CDRs (KABAT) believed to correspond to the antigen binding region are marked.   Also marked are a number of other residues which may also contribute to antigen binding as described in 13.1(c).   Above the sequence in Figure 3 the residue type indicates the spatial location of each residue side chain, derived by examination of resolved structures from X-ray crystallography analysis.   The key to this residue type designation is as follows:

N - near to CDR (From X-ray Structures)

P - Packing   B - Buried Non-Packing

S - Surface   E - Exposed

I - Interface   * - Interface

  - Packing/Part Exposed

? - Non-CDR Residues which may require to be left as Mouse sequence.

Residues underlined in Figure 3 are amino acids. RE1 was chosen as the human framework because the light chain is a kappa chain and the kappa variable regions show higher homology with the mouse sequences than a lambda light variable region, e.g. KOL (see below).   RE1 was chosen in preference to another kappa light chain because the X-ray structure of the light chain has been determined so that a structural examination of individual residues could be made.

12.1.2.   HEAVY CHAIN

Similarly Figure 4 shows an alignment of sequences for the human framework region KOL and the OKT3 heavy variable region.   The structural loops and CDRs believed to correspond to the

antigen binding region are marked. Also marked are a number of other residues which may also contribute to antigen binding as described in 12.1(c). The residue type key and other indicators used in Figure 4 are the same as those used in Figure 3.

KOL was chosen as the heavy chain framework because the X-ray structure has been determined to a better resolution than, for example, NEWM and also the sequence alignment of OKT3 heavy variable region showed a slightly better homology to KOL than to NEWM.

12.2. DESIGN OF VARIABLE GENES

The variable region domains were designed with mouse variable region optimal codon usage [Grantham and Perrin (ref. 15)] and used the B72.3 signal sequences [Whittle et al (ref. 13)]. The sequences were designed to be attached to the constant region in the same way as for the chimeric genes described above. Some constructs contained the "Kozak consensus sequence" [Kozak (ref. 16)] directly linked to the 5′ of the signal sequence in the gene. This sequence motif is believed to have a beneficial role in translation initiation in eukaryotes.

12.3. GENE CONSTRUCTION

To build the variable regions, various strategies are available. The sequence may be assembled by using oligonucleotides in a manner similar to Jones et al (ref. 17) or by simultaneously replacing all of the CDRs or loop regions by oligonucleotide directed site specific mutagenesis in a manner similar to Verhoeyen et al (ref. 2). Both strategies were used and a list of constructions is set out in Tables 1 and 2 and Figures 4 and 5. It was noted in several

cases that the mutagenesis approach led to
deletions and rearrangements in the gene being
remodelled, while the success of the assembly
approach was very sensitive to the quality of the
oligonucleotides.

13.   CONSTRUCTION OF EXPRESSION VECTORS
Genes were isolated from M13 or SP65 based
intermediate vectors and cloned into pEE6hCMVneo
for the light chains and pEE6hCMVgpt for the
heavy chains in a manner similar to that for the
chimeric genes as described above.

	...inclusive	SDM and gene assembly	+	n.d.
121A	26-32, 50-56, 91-96 inclusive +1, 3, 46, 47	Partial gene assembly	n.d.	+
121B	26-32, 50-56, 91-96 inclusive + 46, 47	Partial gene assembly	n.d.	+
221	24-24, 50-56, 91-96 inclusive	Partial gene assembly	+	+
221A	24-34, 50-56, 91-96 inclusive +1, 3, 46, 47	Partial gene assembly	+	+
221B	24-34, 50-56, 91-96 inclusive +1, 3	Partial gene assembly	+	+
221C	24-34, 50-56, 91-96 inclusive	Partial gene assembly	+	+

HEAVY CHAIN	ALL HUMAN FRAMEWORK KOL			
121	26-32, 50-56, 95-100E inclusive	Gene assembly	n.d.	+
131	26-32, 50-58, 95-100B inclusive	Gene assembly	n.d.	+
141	26-32, 50-65, 95-100B inclusive	Partial gene assembly	+	n.d.
321	26-35, 50-56, 95-100B inclusive	Partial gene assembly	+	n.d.
331	26-35, 50-58, 95-100B inclusive	Partial gene assembly	+	
341	26-35, 50-65, 95-100B inclusive	Gene assembly		+
		SDM	+	
		Partial gene assembly		+
341A	26-35, 50-65, 95-100B inclusive +6, 23, 24, 48, 49, 71, 73, 76, 78, 88, 91 (+63 = human)	Gene assembly	n.d.	+
341B	26-35, 50-65, 95-100B inclusive + 48, 49, 71, 73, 76, 78, 88, 91 (+63 + human)	Gene assembly	n.d.	+

KEY

n.d.	not done
SDM	Site directed mutagenesis
Gene assembly	Variable region assembled entirely from oligonucleotides
Partial gene assembly	Variable region assembled by combination of restriction fragments either from other genes originally created by SDM and gene assembly or by oligonucleotide assembly of part of the variable region and reconstruction with restriction fragments from other genes originally created by SDM and gene assembly

14.   EXPRESSION OF CDR-GRAFTED GENES

14.1.   PRODUCTION OF ANTIBODY CONSISTING OF GRAFTED LIGHT
           (gL) CHAINS WITH MOUSE HEAVY (mH) OR CHIMERIC
           HEAVY (cH) CHAINS

           All gL chains, in association with mH or cH
           produced reasonable amounts of antibody.
           Insertion of the Kozak consensus sequence at a
           position 5′ to the ATG (kgL constructs) however,
           led to a 2-5 fold improvement in net
           expression.   Over an extended series of
           experiments expression levels were raised from
           approximately 200ng/ml to approximately 500 ng/ml
           for kgL/cH or kgL/mH combinations.

           When direct binding to antigen on HUT 78 cells
           was measured, a construct designed to include
           mouse sequence based on loop length (gL121) did
           not lead to active antibody in association with
           mH or cH.   A construct designed to include mouse
           sequence based on Kabat CDRs (gL221) demonstrated
           some weak binding in association with mH or cH.
           However, when framework residues 1, 3, 46, 47
           were changed from the human to the murine OKT3
           equivalents based on the arguments outlined in
           Section 12.1 antigen binding was demonstrated
           when both of the new constructs, which were
           termed 121A and 221A were co-expressed with cH.
           When the effects of these residues were examined
           in more detail, it appears that residues 1 and 3
           are not major contributing residues as the
           product of the gL221B gene shows little
           detectable binding activity in association with
           cH.   The light chain product of gL221C, in which
           mouse sequences are present at 46 and 47, shows
           good binding activity in association with cH.

14.2   PRODUCTION OF ANTIBODY CONSISTING OF GRAFTED HEAVY (gH) CHAINS WITH MOUSE LIGHT (mL) OR CHIMERIC LIGHT (cL) CHAINS

Expression of the gH genes proved to be more difficult to achieve than for gL.   First, inclusion of the Kozak sequence appeared to have no marked effect on expression of gH genes. Expression appears to be slightly improved but not to the same degree as seen for the grafted light chain.

Also, it proved difficult to demonstrate production of expected quantities of material when the loop choice (amino acid 26-32) for CDR1 is used, e.g. gH121, 131, 141 and no conclusions can be drawn about these constructs.

Moreover, co-expression of the gH341 gene with cL or mL has been variable and has tended to produce lower amounts of antibody than the cH/cL or mH/mL combinations.   The alterations to gH341 to produce gH341A and gH341B lead to improved levels of expression.

This may be due either to a general increase in the fraction of mouse sequence in the variable region, or to the alteration at position 63 where the residue is returned to the human amino acid Valine (Val) from Phenylalanine (Phe) to avoid possible internal packing problems with the rest of the human framework.   This arrangement also occurs in gH331 and gH321.

When gH321 or gH331 were expressed in association with cL, antibody was produced but antibody binding activity was not detected.

When the more conservative gH341 gene was used antigen binding could be detected in association with cL or mL, but the activity was only marginally above the background level.

When further mouse residues were substituted based on the arguments in 12.1, antigen binding could be clearly demonstrated for the antibody produced when kgH341A and kgH341B were expressed in association with cL.

14.3   PRODUCTION OF FULLY CDR-GRAFTED ANTIBODY

The kgL221A gene was co-expressed with kgH341, kgH341A or kgH341B.   For the combination kgH221A/kgH341 very little material was produced in a normal COS cell expression.

For the combinations kgL221A/kgH341A or kgH221A/kgH341B amounts of antibody similar to gL/cH was produced.

In several experiments no antigen binding activity could be detected with kgH221A/gH341 or kgH221A/kgH341 combinations, although expression levels were very low.

Antigen binding was detected when kgL221A/kgH341A or kgH221A/kgH341B combinations were expressed. In the case of the antibody produced from the kgL221A/kgH341A combination the antigen binding was very similar to that of the chimeric antibody.

An analysis of the above results is given below.

15.   <u>DISCUSSION OF CDR-GRAFTING RESULTS</u>

In the design of the fully humanised antibody the aim was to transfer the minimum number of mouse amino acids that would confer antigen binding onto a human antibody framework.

15.1.   LIGHT CHAIN

15.1.1.   EXTENT OF THE CDRs

For the light chain the regions defining the loops known from structural studies of other antibodies to contain the antigen contacting residues, and those hypervariable sequences

defined by Kabat et al (refs. 4 and 5) as
Complementarity Determining Regions (CDRs) are
equivalent for CDR2. For CDR1 the hypervariable
region extends from residues 24-34 inclusive
while the structural loop extends from 26-32
inclusive. In the case of OKT3 there is only
one amino acid difference between the two
options, at amino acid 24, where the mouse
sequence is a serine and the human framework RE1
has glutamine. For CDR3 the loop extends from
residues 91-96 inclusive while the Kabat
hypervariability extends from residues 89-97
inclusive. For OKT3 amino acids 89, 90 and 97
are the same between OKT3 and RE1 (Fig. 3).
When constructs based on the loop choice for CDR1
(gL121) and the Kabat choice (gL221) were made
and co-expressed with mH or cH no evidence for
antigen binding activity could be found for
gL121, but trace activity could be detected for
the gL221, suggesting that a single extra mouse
residue in the grafted variable region could have
some detectable effect. Both gene constructs
were reasonably well expressed in the transient
expression system.

15.1.2. FRAMEWORK RESIDUES

The remaining framework residues were then
further examined, in particular amino acids known
from X-ray analysis of other antibodies to be
close to the CDRs and also those amino acids
which in OKT3 showed differences from the
consensus framework for the mouse subgroup
(subgroup VI) to which OKT3 shows most
homology. Four positions 1, 3, 46 and 47 were
identified and their possible contribution was
examined by substituting the mouse amino acid for
the human amino acid at each position.

Therefore gL221A (gL221 + D1Q, Q3V, L46R, L47W, see Figure 3 and Table 1) was made, cloned in EE6hCMVneo and co-expressed with cH (pJA144). The resultant antibody was well expressed and showed good binding activity. When the related genes gL221B (gL221 + D1Q, Q3V) and gL221C (gL221 + L46R, L47W) were made and similarly tested, while both genes produced antibody when co-expressed with cH, only the gL221C/cH combination showed good antigen binding. When the gL121A (gL121 + D1Q, Q3V, L46R, L47W) gene was made and co-expressed with cH, antibody was produced which also bound to antigen.

15.2.   HEAVY CHAIN

15.2.1.   EXTENT OF THE CDRs

For the heavy chain the loop and hypervariability analyses agree only in CDR3. For CDR1 the loop region extends from residues 26-32 inclusive whereas the Kabat CDR extends from residues 31-35 inclusive. For CDR2 the loop region is from 50-58 inclusive while the hypervariable region covers amino acids 50-65 inclusive. Therefore humanised heavy chains were constructed using the framework from antibody KOL and with various combinations of these CDR choices, including a shorter choice for CDR2 of 50-56 inclusive as there was some uncertainty as to the definition of the end point for the CDR2 loop around residues 56 to 58. The genes were co-expressed with mL or cL initially. In the case of the gH genes with loop choices for CDR1 e.g. gH121, gH131, gH141 very little antibody was produced in the culture supernatants. As no free light chain was detected it was presumed that the antibody was being made and assembled inside the cell but that the heavy chain was aberrant in

some way, possibly incorrectly folded, and
therefore the antibody was being degraded
internally.   In some experiments trace amounts
of antibody could be detected in ^{35}S labelling
studies.

As no net antibody was produced, analysis of
these constructs was not pursued further.

When, however, a combination of the loop choice
and the Kabat choice for CDR1 was tested (mouse
amino acids 26-35 inclusive) and in which
residues 31 (Ser to Arg), 33 (Ala to Thr), and 35
(Tyr to His) were changed from the human residues
to the mouse residue and compared to the first
series, antibody was produced for gH321, kgH331
and kgH341 when co-expressed with cL.

Expression was generally low and could not be
markedly improved by the insertion of the Kozak
consensus sequence 5' to the ATG of the signal
sequence of the gene, as distinct from the case
of the gL genes where such insertion led to a 2-5
fold increase in net antibody production.

However, only in the case of gH341/mL or
kgH341/cL could marginal antigen binding activity
be demonstrated.   When the kgH341 gene was
co-expressed with kgL221A, the net yield of
antibody was too low to give a signal above the
background level in the antigen binding assay.

15.2.2. FRAMEWORK RESIDUES

As in the case of the light chain the heavy chain
frameworks were re-examined.   Possibly because
of the lower initial homology between the mouse
and human heavy variable domains compared to the
light chains, more amino acid positions proved to
be of interest.   Two genes kgH341A and kgH341B
were constructed, with 11 or 8 human residues
respectively substituted by mouse residues

compared to gH341, and with the CDR2 residue 63 returned to the human amino acid potentially to improve domain packing. Both showed antigen binding when combined with cL or kgL221A, the kgH341A gene with all 11 changes appearing to be the superior choice.

15.3 INTERIM CONCLUSIONS

It has been demonstrated, therefore, for OKT3 that to transfer antigen binding ability to the humanised antibody, mouse residues outside the CDR regions defined by the Kabat hypervariability or structural loop choices are required for both the light and heavy chains. Fewer extra residues are needed for the light chain, possibly due to the higher initial homology between the mouse and human kappa variable regions.

Of the changes seven (1 and 3 from the light chain and 6, 23, 71, 73 and 76 from the heavy chain) are predicted from a knowledge of other antibody structures to be either partly exposed or on the antibody surface. It has been shown here that residues 1 and 3 in the light chain are not absolutely required to be the mouse sequence; and for the heavy chain the gH341B heavy chain in combination with the 221A light chain generated only weak binding activity. Therefore the presence of the 6 and 23 and 24 changes are important to maintain binding affinity similar to that of murine antibody. It was important, therefore, to further study the individual contribution of the other 8 mouse residues of the kgH341A gene compared to kgH341.

16. FURTHER CDR-GRAFTING EXPERIMENTS

Additional CDR-grafted heavy chain genes were prepared substantially as described above. With

reference to Table 2 the further heavy chain
genes were based upon the gh341 (plasmid pJA178)
and gH341A (plasmid pJA185) with either mouse
OKT3 or human KOL residues at 6, 23, 24, 48, 49,
63, 71, 73, 76, 78, 88 and 91, as indicated.
The CDR- grafted light chain genes used in these
further experiments were gL221, gL221A, gL221B
and gL221C as described above.

TABLE 2

OKT3 HEAVY CHAIN CDR GRAFTS

1.   gH341 and derivatives

RES NUM	6	23	24	48	49	63	71	73	76	78	88	91	
OKT3vh	Q	K	A	I	G	F	T	K	S	A	A	Y	
gH341	.E	S	S	V	A	F	R	N	N	L	G	F	JA178
gH341A	Q	K	A	I	G	V	T	K	S	A	A	Y	JA185
gH341E	Q	K	A	I	G	V	T	K	S	A	G	G	JA198
gH341*	Q	K	A	I	G	V	T	K	N	A	·G	F	JA207
gH341*	Q	K	A	I	G	V	R	N	N	A	G	F	JA209
gH341D	Q	K	A	I	G	V	T	K	N	L	G	F	JA197
gH341*	Q	K	A	I	G	V	R	N	N	L	G	F	JA199
gH341C	Q	K	A	V	A	F	R	N	N	L	G	F	JA184
gH341*	Q	S	A	I	G	V	T	K	S	A	A	Y	JA203
gH341*	E	S	A	I	G	V	T	K	S	A	A	Y	JA205
gH341B	E	S	S	I	G	V	T	K	S	A	A	Y	JA183
gH341*	Q	S	A	I	G	V	T	K	S	A	G	F	JA204
gH341*	E	S	A	I	G	V	T	K	S	A	G	F	JA206
gH341*	Q	S	A	I	G	V	T	K	N	A	G	F	JA208
KOL	E	S	S	V	A		R	N	N	L	G	F	

OKT3 LIGHT CHAIN CDR GRAFTS

2.   gL221 and derivatives

RES NUM	1	3	46	47	
OKT3vl	Q	V	R	W	
GL221	D	Q	L	L	DA221
gL221A	Q	V	R	W	DA221A
gL221B	Q	V	L	L	DA221B
GL221C	D	Q	R	W	DA221C
RE1	D	Q	L	L	

MURINE RESIDUES ARE UNDERLINED

The CDR-grafted heavy and light chain genes were co-expressed in COS cells either with one another in various combinations but also with the corresponding murine and chimeric heavy and light chain genes substantially as described above. The resultant antibody products were then assayed in binding and blocking assays with HPB-ALL cells as described above.

The results of the assays for various grafted heavy chains co-expressed with the gL221C light chain are given in Figures 7 and 8 (for the JA184, JA185, JA197 and JA198 constructs - see Table 2), in Figure 9 (for the JA183, JA184, JA185 and JA197 constructs) in Figure 10 (for the chimeric, JA185, JA199, JA204, JA205, JA207, JA208 and JA209 constructs) and in Figure 11 (for the JA183, JA184, JA185, JA198, JA203, JA205 and JA206 constructs).

The basic grafted product without any human to murine changes in the variable frameworks, i.e. gL221 co-expressed with gh341 (JA178), and also the "fully grafted" product, having most human to murine changes in the grafted heavy chain framework, i.e. gL221C co-expressed with gh341A (JA185), were assayed for relative binding affinity in a competition assay against murine OKT3 reference standard, using HPB-ALL cells. The assay used was as described above in section 3.3. The results obtained are given in Figure 12 for the basic grafted product and in Figure 13 for the fully grafted product. These results indicate that the basic grafted product has relatively poor binding abiliaty as compared with the OKT3 murine reference standard; whereas the "fully grafted" product has a binding ability very similar to that of the OKT3 murine reference standard.

The binding and blocking assay results indicate the following:

The JA198 and JA207 constructs appear to have the best binding characteristics and similar binding abilities, both substantially the same as the chimeric and fully grafted gH341A products. This indicates that positions 88 and 91 and position 76 are not highly critical for maintaining the OKT3 binding ability; whereas at least some of positions 6, 23, 24, 48, 49, 71, 73 and 78 are more important.

This is borne out by the finding that the JA209 and JA199, although of similar binding ability to one another, are of lower binding ability than the JA198 and JA207 constructs. This indicates the importance of having mouse residues at positions 71, 73 and 78, which are either completely or partially human in the JA199 and JA209 constructs respectively.

Moreover, on comparing the results obtained for the JA205 and JA183 constructs it is seen that there is a decrease in binding going from the JA205 to the JA183 constructs. This indicates the importance of retaining a mouse residue at position 23, the only position changed between JA205 and JA183.

These and other results lead us to the conclusion that of the 11 mouse framework residues used in the gH341A (JA185) construct, it is important to retain mouse residues at all of positions 6, 23, 24, 48 and 49, and possibly for maximum affinity at positions 71, 73 and 78.

REFERENCES

1. Kohler & Milstein, Nature, <u>265</u>, 295-497, 1975.

2. Verhoeyen <u>et al</u>, Science, <u>239</u>, 1534-1536, 1988.

3. Riechmann <u>et al</u>, Nature, <u>332</u>, 323-324, 1988.

4. Kabat, E.A., Wu, T.T., Reid-Miller, M., Perry, H.M., Gottesman, K.S., 1987, in Sequences of Proteins of Immunological Interest, US Department of Health and Human Services, NIH, USA.

5. Wu, T.T., and Kabat, E.A., 1970, J. Exp. Med. <u>132</u> 211-250.

6. Queen <u>et al</u>, (1989), Proc. Natl. Acad. Sci. USA, <u>86</u>, 10029-10033 and WO 90/07861

7. Chatenoud <u>et al</u>, (1986), J. Immunol. <u>137</u>, 830-838.

8. Jeffers <u>et al</u>, (1986), Transplantation, <u>41</u>, 572-578.

9. Maniatis <u>et al</u>, Molecular Cloning, Cold Spring Harbor, New York, 1982.

10. Primrose and Old, Principles of Gene Manipulation, Blackwell, Oxford, 1980.

11. Sanger, F., Nicklen, S., Coulson, A.R., 1977, Proc. Natl. Acad. Sci. USA, <u>74</u> 5463

12. Kramer, W., Drutsa, V., Jansen, H.-W., Kramer, B., Plugfelder, M., Fritz, H.-J., 1984, Nucl. Acids Res. <u>12</u>, 9441

13. Whittle, N., Adair, J., Lloyd, J.C., Jenkins, E.,
Devine, J., Schlom, J., Raubitshek, A., Colcher, D.,
Bodmer, M., 1987, Protein Engineering $\underline{1}$, 499.

14. Bebbington, C.R., Published International Patent
Application WO 89/01036.

15. Granthan and Perrin 1986, Immunology Today $\underline{7}$, 160.

16. Kozak, M., 1987, J. Mol. Biol. $\underline{196}$, 947.

17. Jones, T.P., Dear, P.H., Foote, J., Neuberger, M.S.,
Winter, G., 1986, Nature, $\underline{321}$, 522

CLAIMS

1. A recombinant antibody molecule comprising antigen
    binding regions derived from the heavy and/or light
    chain variable regions of a donor anti-CD3 antibody
    and having an anti-CD3 binding specificity.

2. A recombinant antibody molecule according to Claim 1,
    having an anti-CD3 binding affinity similar to that
    of OKT3.

3. A recombinant antibody molecule according to Claim 1
    or Claim 2 which is a chimeric antibody.

4. A recombinant antibody molecule according to Claim 1
    or Claim 2 which is a CDR-grafted antibody.

5. A recombinant antibody molecule according to any of
    the preceding claims which is a humanised antibody
    molecule.

6. A CDR-grafted antibody heavy chain according to Claim
    4, having a variable region domain comprising
    acceptor framework and donor CD3 binding regions
    wherein the framework comprises donor residues at at
    least one of positions 6, 23 and/or 24, 48 and/or 49,
    71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91.

7. A CDR-grafted heavy chain according to Claim 6
    comprising donor residues at positions 23, 24, 49,
    71, 73 and 78, or at positions 23, 24 and 49.

8. A CDR-grafted heavy chain according to Claim 7
    comprising donor residues at positions 2, 4, 6, 25,
    36, 37, 39, 47, 48, 93, 94, 103, 104, 106 and 107.

9. A CDR-grafted heavy chain according to Claim 7 or 8, comprising donor residues at one, some or all of positions:

    1 and 3,

    69 (if 48 is different between donor and acceptor),

    38 and 46 (if 48 is the donor residue),

    67,

    82 and 18 (if 67 is the donor residue),

    91, and

    any one or more of 9, 11, 41, 87, 108, 110 and 112.

10. A CDR-grafted heavy chain according to any one of Claims 4, or 6-9 comprising donor CDRs at positions 26-35, 50-65 and 95-100.

11. A CDR-grafted antibody light chain according to Claim 4, having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 1 and/or 3 and 46 and/or 47.

12. A CDR-grafted light chain according to Claim 11 comprising donor residues at positions 46 and 47.

13. A CDR-grafted antibody light chain according to Claim 4, having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 46, 48, 58 and 71.

14. A CDR-grafted light chain according to Claim 13 comprising donor residues at positions 46, 48, 58 and 71.

15. A CDR-grafted light chain according to Claim 11 or 13, comprising donor residues at positions 2, 4, 6, 35, 36, 38, 44, 47, 49, 62, 64-69, 85, 87, 98, 99, 101 and 102.

16. A CDR-grafted light chain according to any one of Claims 11, 13, or 15, comprising donor residues at one, some or all of positions:
1 and 3,
63,
60 (if 60 and 54 are able to form a potential saltbridge),
70 (if 70 and 24 are able to form a potential saltbridge),
73 and 21 (if 47 is different between donor and acceptor),
37 and 45 (if 47 if different between donor and acceptor), and
any one or more of 10, 12, 40, 83, 103 and 105.

17. A CDR-grafted light chain according to any one of Claims 4 or 11-16, comprising donor CDRs at positions 24-34, 50-56 and 89-97.

18. A CDR-grafted antibody molecule comprising at least one CDR-grafted heavy chain according to any one of Claims 6-10 and at least one CDR-grafted light chain according to any one of Claims 11-17.

19. A CDR-grafted antibody heavy or light chain or molecule according to any one of Claims 6-18 comprising human acceptor residues and non-human donor residues.

20. A DNA sequence which codes for a recombinant antibody according to Claim 1, a humanised antibody according to Claim 5, a CDR-grafted heavy chain according to Claim 6 or a CDR-grafted light chain according to Claim 11 or Claim 13.

21. A cloning or expression vector containing a DNA sequence according to Claim 20.

22. A host cell transformed with a DNA sequence according to Claim 20.

23. A process for the production of an anti-CD3 CDR-grafted antibody product comprising expressing a DNA sequence according to Claim 20 in a transformed host cell.

24. A process for producing an anti-CD3 CDR-grafted antibody product comprising:
   (a) producing in an expression vector an operon having a DNA sequence which encodes an antibody heavy chain according to Claim 6;
   and/or
   (b) producing in an expression vector an operon having a DNA sequence which encodes a complementary antibody light chain according to Claim 11 or Claim 13;
   (c) transfecting a host cell with the or each vector; and
   (d) culturing the transfected cell line to produce the CDR-grafted antibody product.

25. A therapeutic or diagnostic composition comprising a recombinant antibody molecule according to Claim 1, a humanised antibody molecule according to Claim 5, a CDR-grafted antibody heavy chain according to Claim 6,

a CDR-grafted light chain according to Claim 11 or Claim 13, or a CDR-grafted antibody molecule according to Claim 18 in combination with a pharmaceutically acceptable carrier, diluent or excipient.

26. A method of therapy or diagnosis comprising administering an effective amount of a recombinant antibody molecule according to Claim 1, a humanised antibody molecule according to Claim 5, a CDR-grafted heavy chain according to Claim 6, or a CDR-grafted light chain according to Claim 11 or Claim 13, or a CDR-grafted antibody molecule according to Claim 18 to a human or animal subject.